CONJOINT *HELIGMOSOMOIDES POLYGYRUS* AND *TRYPANOSOMA CONGOLENSE* INFECTION IN MICE

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DECLARATION

I hereby declare that the work presented in this thesis is the product of own efforts, except where specifically stated in the text and in the acknowledgements section. No part of this thesis has been submitted in any previous application for a degree.

BB Fakae

This thesis is dedicated to The Fakaes of Kabangha and to the glory of God.

'A righteous man cares for the needs of his animal, but the kindest acts of the wicked are cruel.' (Proverbs 12:10 NIV).

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ABBREVIATIONS AND SYMBOLS

g	Acceleration due to gravity
BBS	Borate buffered saline
CTVM	Centre for Tropical Veterinary Medicine
CTAB	Cetyltrimethyl-ammonium bromide
DAI	Days after infection
°C	Degrees centigrade
DE-52	Diethylaminoethyl cellulose
DMSO	Dimethyl Sulphoxide
EPG	Eggs per gram of faeces
ELISA	Enzyme-linked immunosorbent assay
FCS	Foetal calf serum
IFN-γ	Gamma interferon
GAM	Goat anti-mouse
g	Gram
HBBS	Hanks balanced salt solution
H&E	Haematoxylin and eosin
HEPES	4-2(2-Hydroxyethyl)-1-piperazine ethane
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
kD	Kilodalton
Kg	Kilogram
log	Logarithm to base 10
MHC	Major histocompatibility complex
mVAT	Metacyclic variable antigenic type
μg	Microgram
μ	Micron
mg	Milligram
ml	Millilitre
mm	Millimeter
mM	Millimolar
nm	Nanometer
NOG	n-octyl-B-d-glucopyranoside
NGS	Normal goat serum
PCV	Packed cell volume
PAS	Periodic Acid-Schiff
PO	Peroxidase
PBS	Phosphate buffered saline
PSG	Phosphate buffered glucose saline
Tween 20	Polyoxyethylene sortitan monolaurate
P	Probability
PI	Protease Inbibitors
NaCl	Sodium chloride
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SRBC	Sheep red blood cells
SG	Specific gravity
SEM (SE)	Standard error of mean
TH	T helper cells
H_2SO_4	Sulphoric acid
TREU	Trypanosome Research Edinburgh University
TO	Tuck Outbred
VAT	Variable antigenic type
VSG	Varian specific glycoprotein
v/v	Volume by volume
w/v	Weight by volume
WBC	White blood cells

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SUMMARY

The economic importance of gastro-intestinal nematodes and African trypanosome infections of farm animals in the tropics has been presented as the rationale for research into the aspects of these infections with the hope of devising control measures which will increase animal productivity. The scientific literature on the development, immunology and pathology of gastro-intestinal nematode and protozoan infections with particular reference to *Heligmosomoides polygyrus* and African trypanosomes and the effects of concurrent infections, with these classes of parasites, on the host has been reviewed.

Experiments were conducted to develop a suitable laboratory model involving *H. polygyrus* and *Trypanosoma congolense* in female TO mice in order to study the effects of some factors such as the timing of conjoint infections, the role of immunity to the helminth infection in the face of conjoint challenge infection and the responses of the host as well as that of the parasites to conjoint infections.

Infections with stabilated blood stream forms of *T. congolense* (TREU 1881) but not the infective metacyclics grown *in vitro* gave reproducible parasitological results which together with infections of 500 L_3 of *H. polygyrus* in mice produced a suitable host-parasite system in which aspects of chronic gastro-intestinal nematode and blood protozoan infections were studied.

Except when *T. congolense* was superimposed on a 10-day old *H. polygyrus* infection, mice conjointly infected with *T. congolense* during a primary *H. polygyrus* infection were severely compromised, resulting in enhanced mortality. The synergistic pathogenic effects of dual infections in mice were particularly marked when *T. congolense* infection preceded infection with the nematode. *T. congolense* infection depressed the immune (cellular and humoral) responses which normally occur in mice after primary *H. polygyrus* infection. The protozoan infection either reduced or totally inhibited immunity against a challenge *H. polygyrus* infection. Possible mechanisms of this immunosuppression which include among others, the generation of suppressor macrophages and the inhibition

of eosinophilia, are discussed. These observations suggest that, conjoint infections with these parasites produce deleterious synergistic interactions which affect productivity and even cause further deaths.

Although the protective responses against homologous challenge in mice immunized by abbreviation of *H. polygyrus* adult infection were completely lost as a result of concurrent infection with *T. congolense*, the stronger protection in those immunized by an abbreviated larval infection was merely reduced. These observations suggest that animals with a strong immunity to gastro-intestinal nematodes may largely overcome the suppressive influence of the trypanosome. It is suggested that enhanced resistance through abbreviation of primary nematode infection within the first one week of grazing an infected pasture at the beginning of the grazing season might combat problems of synergistic interactions during natural infections of nematodes and trypanosomes in ruminants.

CHAPTER ONE

INTRODUCTION

Gastrointestinal nematodes have been recognized as a major cause of impaired productivity in ruminants (Chiejina, 1986, Fabiyi, 1987). In some tropical countries, such as Nigeria, the prevalence of nematode infections in small ruminants can be as high as 77-100% throughout the year without any seasonal variation (Fakae, 1990a, 1990b). These infections may be sub-clinical, leading to chronic infections (Chiejina, 1987) or outbreaks with heavy mortalities (Fabiyi, Oluyede and Negedu, 1979). Where these infections are not controlled they may have devastating consequences on animal productivity (Schillhorn van Veen, 1973).

Loss of productivity due to such disease has been shown to be derived from various pathophysiological effects on the parasitized animals (Holmes, 1987). One of the most common signs of infection is a reduction in the liveweight gain of the affected animals. However, the rate and extent of such loss may be modified by various factors such as age, sex, immune status of the host, lactation, nutrition and level of parasite burden, as well as the nematode species involved (Bawden, 1969; Thomas and Ali, 1983; Gruner and Cabaret, 1988; Pomroy and Charleston, 1989b; Blackburn, Rocha, Figueiredo, Berne, Cavalcante and Rosa, 1991). Some controlled studies have shown that up to 60% weight loss may occur in sheep infected with *Trichostrongylus colubriformis* or *Ostertagia circumcincta* (Sykes and Coop, 1976, 1977). Various treatment experiments have shown that the termination of such infection by anthelmintics results in a substantial weight gain in animals (Chiejina and Emehelu, 1986).

In addition to the loss of weight, infected animals also give very poor carcase quality. This may be due to the decrease in the deposition of fat and protein, as well as skeletal mineralization and relatively high percentage body water content (Reveron, Topps and Gelman, 1974; Sykes and Coop, 1976, 1977; Sykes, Coop and Angus, 1977).

In dairy cows, the economic impact of gastrointestinal helminth infection is illustrated in studies such as those of Bliss and Todd (1976), where anthelmintic

treatment was shown to increase milk yield. Some results obtained from sheep have also indicated that infection resulted in low milk yield (Thomas and Ali, 1983; Sykes and Juma, 1984)

In sheep from temperate climate where wool is important, the quality and quantity of wool may be adversely affected by gastrointestinal nematode infection (Steel, Jones and Symons, 1982). Even immune sheep which are under continuous helminth challenge have been shown to incur a production penalty in terms of wool growth (Steel, Symons and Jones, 1980; Wagland, Steel, Windon and Dineen, 1984).

Production losses vary, depending on the part of the world and the system of animal husbandry. Bain and Urquhart (1986) estimated that the cost of uncontrolled helminth infection to UK farmers could be as high as £45 million annually. In heavily stocked farms in subtropical countries, production losses are generally thought to be higher. For instance, mortality due to infection could account for losses up to 30% of the stock (Vassiliades, 1974; Eysker and Ogunsusi, 1980). In most of these countries economic losses due to helminthosis are difficult to estimate accurately because of lack of relevant data, since most subclinical and chronic infections are rarely diagnosed in the rural setting, while the clinical cases that are diagnosed are poorly documented. However, Akerejola, Schillhorn van Veen and Njoku (1979) conservatively estimated that, in Nigeria, the annual loss due to helminthosis in small ruminants alone is at least US.$60 million. The economic losses due to these infections have not been properly assessed as they are often derived only or mostly by comparing the effects of overt clinical cases with other diseases, without taking into cognizance the widespread effect of the subclinical parasitism (Sykes, 1978) which characterises these infections.

In sub-Saharan Africa, trypanosomosis is another major disease that has continued to pose serious problems to the animal industries (WHO, 1979). Profitable production of livestock is limited by trypanosomosis through decreased milk yield, abortion, unsatisfactory growth rate and mortality (Griffin and

Allonby, 1979; Ikede, Elhassan and Akpavie, 1988; Edeghere, Elhassan, Abenga, Osue, Lawani and Falope, 1992). Infected animals that are salvaged show very poor carcase quality due to extreme emaciation.

The insidious nature of trypanosomosis in those indigenous breeds which are believed to be trypanotolerant tend to present a false sense of wellbeing, as infections can go unnoticed. Despite this trypanotolerance, intercurrent factors such as pregnancy, lactation and a low plane of nutrition may lead to unacceptable consequences as animals become susceptible to the infection (Reynolds and Ekwuruke, 1988; Fagbemi, Otesile, Makinde and Akinboade, 1990). Not only do the animals suffer from the infection but they also serve as potential reservoirs for other animals (Killick-Kendrick and Godfrey 1963; Mahmound and Elmalik, 1977) and possibly humans (Scott, Frezil, Toudic and Godfrey, 1983).

At present, the control of each of these disease entities is planned separately. Accordingly the possible effects of conjoint nematode-trypanosome infections on treatment and control programmes are not yet clear, although Stephen (1970) observed increased virulence of *T. brucei* or enhanced toxicity of an anthelmintic (phenothiazine) when conjointly infected cattle were treated. It is therefore clear that an understanding of the interaction between the two parasites and their hosts and the resultant effect on the hosts' productivity and response to treatment will be of direct relevance to economy of livestock production.

Although helminthosis and trypanosomosis are both known to be endemic in the tropics, most studies and surveys have examined these diseases as separate entities, thus ignoring the fact that they frequently occur naturally as conjoint infections. Such dual infections may modify the course of each individual infection if the hosts are more severely compromised by the concurrent infections. The only available studies in small ruminants showed that, in two breeds of goats, the interaction between *T. congolense* and *Haemonchus contortus* resulted in greater pathological effects than in the single infections (Griffin, Allonby and Preston 1981a; Griffin, Aucutt, Allonby, Preston and Castelino, 1981b). This has been

confirmed very recently in trypanotolerant N'Dama cattle in the Gambia by Kaufmann, Dwinger, Hallebeek, van Dijk and Pfister (1992). As observed by Chiejina (1987) and Fakae and Chiejina (1993), low levels of trypanosomes often coexist with heavy nematode infection in West African Dwarf sheep and goats which is suggestive of a synergistic modulation of the sustainability of the worm infection possibly due to a reduced capacity of the animal to respond immunologically.

Studies of helminth-protozoan interactions in small laboratory animals have shown that there can be enhancement of survival and pathogenicity of one or both parasites (Phillips, Selby and Wakelin, 1974; Bell, Adams and Ogden, 1984a,b; Nichol and Sewell, 1984;). Such has also been found to be true of concomitant nematode and virus infections in mice (Chowaniec, Wescott and Congdon, 1972). However, there are also contrasting well-documented demonstrations of antagonistic interactions between protozoan and helminths in concurrent experimental infections in the laboratory mouse (Christensen, Furu, Kurtzhals and Odaibo, 1988; Fagbemi, Christensen and Nansen, 1985a, 1985b; Christensen, Nansen, Fagbemi and Monrad, 1987; Phillips and Wakelin, 1976). Since the outcome of conjoint infection may differ according to the parasites involved, care is needed when deciding on parasites to be used in models which are intended to simulate infections in farm animals.

The use of large farm animals for such fundamental study is known to be very expensive. The few related models that have been developed in rodents have used nematodes which are very short lived in the host (Urquhart, Murray, Murray, Jennings and Bate, 1973) or trypanosomes that are relatively non-pathogenic and unrelated to the species that occur in ruminants (Bell, Adams and Ogden, 1984a, Albright and Albright, 1991). Although these studies may provide some useful information, the outcomes may be difficult to relate to interactions in the ruminants.

Heligmosomoides polygyrus, however, is known to produce a chronic infection lasting 30-38 weeks in mice (Behnke and Robinson, 1985). Such chronic infections are similar to those of *Haemonchus contortus* and *Trichostrongylus colubriformis* in ruminants which may survive for 30-55 weeks (Allonby and Urquhart, 1973; Adams and Beh, 1981; Chiejina, Fakae and Eze, 1988). This *H. polygyrus* model has been proposed as an excellent model for chronic gastro-intestinal helminthosis of mammals (Bartlett and Ball, 1972; Monroy and Enriquez, 1992). It has so far provided valuable information on the kinetics of larval development (Ey, 1988), the quantitative role of acquired immunity and genetic heterogenicity in helminth population dynamics (Keymer and Hiorns 1986a,b; Wakelin, 1986), mechanisms of immune evasion (Behnke, 1987), and mechanisms of immunodepression against heterologous antigens in conjoint infections (Chowaniec, Wescott and Congdon, 1972; Shimp, Crandall and Crandall, 1975; Jenkins and Behnke, 1977).

As a preliminary step to studying aspects of conjoint nematode and trypanosome infections in ruminants, it was desirable firstly, to develop a suitable and relatively cheap laboratory model. It was decided that this could be done by using a murine trichostrongylid, *H. polygyrus*, which has similar life cycle, chronicity of infection and pathogenicity to some common nematodes of ruminants, in conjunction with *T. congolense*, the most important trypanosome infecting ruminants in the sub-Saharan Africa, which will also readily infect laboratory mice (Losos and Ikede, 1972; Pinder, 1984). Secondly, the model was used to study the effects of some factors such as the timing of conjoint infections, the role of immunity to the helminth infection in the face of conjoint challenge infection and the responses of the host as well as that of the parasites to conjoint infections.

CHAPTER TWO

LITERATURE REVIEW

2.1 *HELIGMOSOMOIDES POLYGYRUS* HALL 1916 (NEMATODA)

Heligmosomoides polygyrus is an intestinal trichostrongylid nematode of microtine and murine rodents which is cosmopolitan in distribution. *H. polygyrus* was first described by Dujardin (1845) as *Strongylus polygyrus*. Since that time, based on descriptions from different observations, several names have been adopted and changed. Until very recently, the parasite was commonly referred to as *Nematospiroides dubius*, a name that was put forward by Baylis (1926). However, Durette-Desset, Kinsella and Forrester (1972) made a strong argument in support of Hall (1916) who had earlier called this same parasite *Heligmosomoides polygyrus*. Asakawa (1988) published a comprehensive review on the genus *Heligmosomoides*, dividing it into five groups on the basis of morphological characteristics, and host distribution.

Recently, Behnke, Keymer and Lewis (1991) published an excellent review of the historical background and the confusion that surrounded the original descriptions of the parasite. They suggested that the laboratory mouse-maintained isolate and all worms derived from the original isolate of Ehrenford (1954) be called *Heligmosomoides polygyrus bakeri.* This original strain, referred to as Strain 50 by Forrester and Neilson (1973), from which the present widely employed laboratory strain originated, was isolated in 1950 from a deer mouse (*Peromyscus maniculatus gambeli*) trapped near Woodland, California, USA (Ehrenford, 1954) and has since then been distributed to laboratories world wide.

Taxonomically, *H. polygyrus bakeri* belongs to the order Strongylida. It is a member of the superfamily, Trichostrongyloidea, family Heligmosomatidae (Dunn, 1978), and the subgroup Polygyrus (Asakawa, 1988).

2.1.1 Morphology

H. polygyrus is a small delicate red worm with a filiform body. Adult males are 6-7mm long and the females 16-19mm long (Panter, 1969; Pritchard, William, Behnke and Lee, 1983; Pritchard and Behnke, 1985; Slater and Keymer, 1986b; East, Washington, Brindly, Monroy and Scott-Young, 1988; Ey, 1988). The mouth is simple and the oesophagus is claviform. The cuticle, which is

pigmented red, has transverse striations and longitudinal ridges (Yamatiguti, 1961; Behnke *et al.*, 1991). The females are coiled into spirals with 12-16 turns and the males, 4-8 turns (Lewis, 1968). It is assumed that the spirals assist in attachment in the gut of the host as the longitudinal striae are embedded into the intestinal villi (Kleinschuster, Hepler and Voth, 1978). The male is bursate and has two long and equal spicules. In the female, the vulva is located anterior to the anus and the tail is conical.

2.1.2 Life Cycle

2.1.2.1 Preparasitic development

Like most members of its superfamily, *H. polygyrus* has a direct life cycle. Ehrenford (1954) first described the life cycle. The egg measures 70-84μ x 37-53μ at the 8-16 cell becomes fully embryonated in 8-10 hours at 23-28°C. According to Ehrenford, there was just one preparasitic moult before oral infection. Fahmy (1956), however, described two preparasitic moults with the first, second and third larval stage measuring 343-365μm, 418-440μm and 443-461μm respectively. It was Bryant (1973) that gave a more precise and detailed account of the life history of the parasite. Under humid condition at 20°C, the first larval stage (L_1) develops in the egg to perform vigorous movements within 28 hours. After 36-37 hours the egg hatches to liberate the L_1. In another 28-29 hours, the L_1 moults to give the second larval stage (L_2) which takes 17-20 hours to undergo a further partial moult to give the third and infective larvae (L_3) which retains the L_2-sheath like the L_3 of the common nematodes of ruminants (Crofton, 1963). Thus preparasitic development can be completed in about four days. However, as with the trichostrongyles of ruminants, successful development and infection depends on a variety of complex and interacting external factors, notably moisture, oxygen tension and temperature (Andersen, Wang and Levine, 1966; McKenna, 1973; Chiejina and Fakae, 1984; Fakae, 1986; Chiejina and Fakae, 1989; Chiejina, Fakae and Eze, 1989). Kerboeuf (1978) observed that the L_3 of *H. polygyrus* cultured at

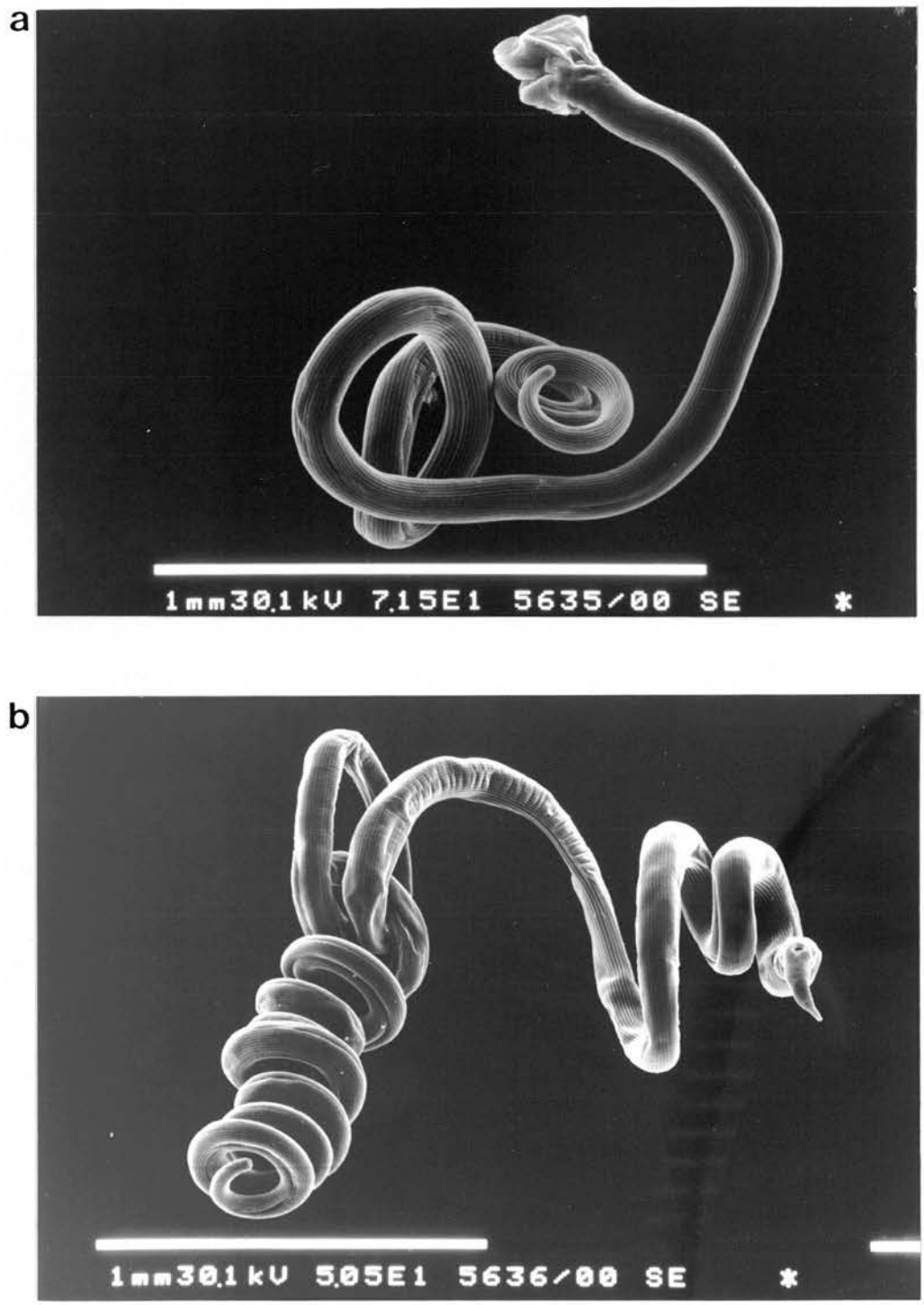

PLATE 2.1 Scanning electron micrograph of adult male (a) and female (b) *Heligmosomoides polygyrus*

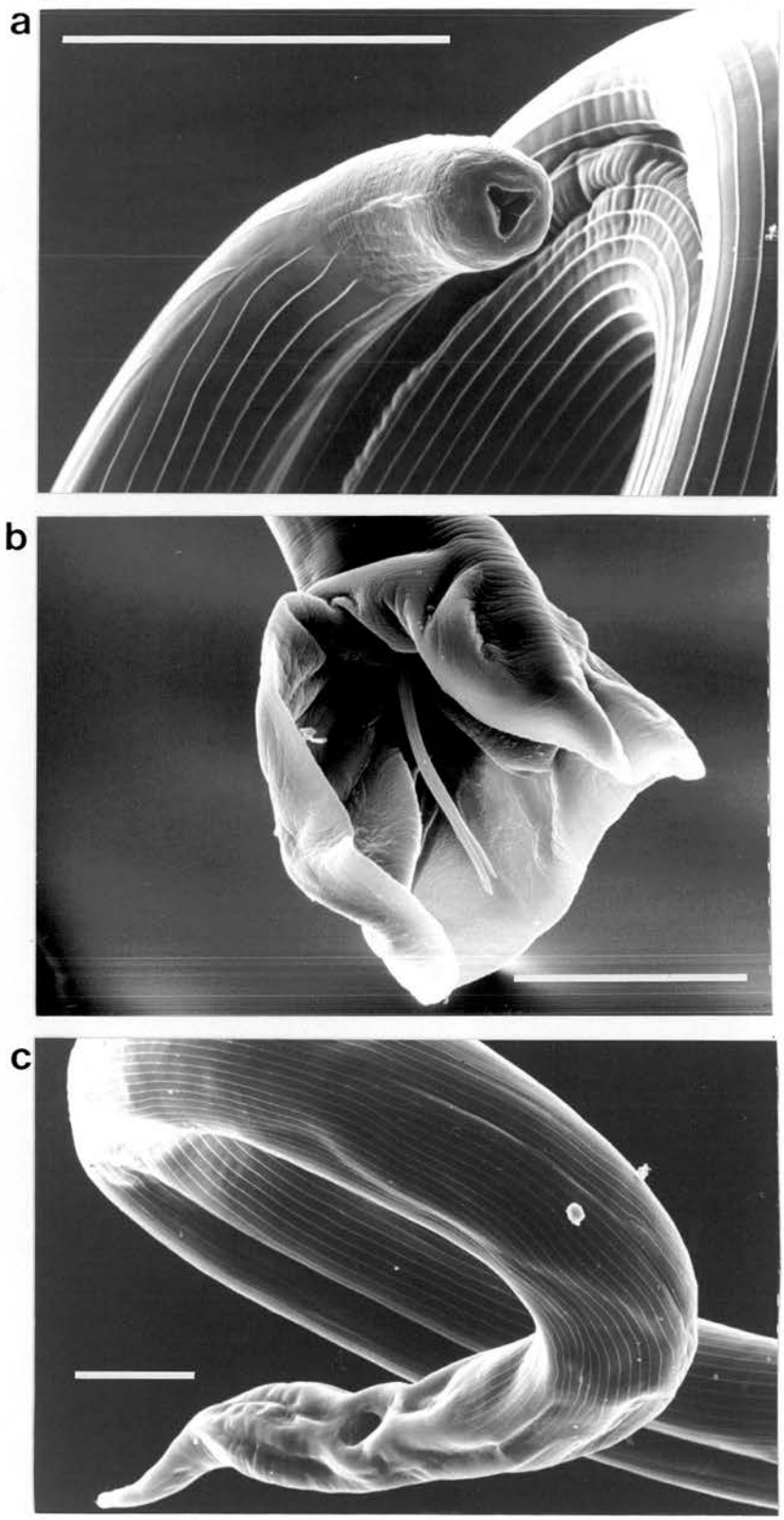

PLATE 2.2 Scanning electron micrograph of head end (a), tail ends of male (b) and female (b) *Heligmosomoides polygyrus* (Scale bar = 1 mm).

22°C and stored at 4°C gave optimum infectivity in mice and that a maturation period was required to obtain maximum infectivity.

2.1.2.2 Parasitic development

Within 24 hours of ingestion by the host, the L_3 exsheaths and unlike *Nippostrongylus brasiliensis* it does not undergo hepatic-tracheal migration but penetrates through the epithelium of the small intestine and develops into the fourth larval stage (L_4) in a cystic nodule beneath the muscularis mucosa. Within 6-8 days from ingestion, the juvenile returns to the intestinal lumen as a sexually mature worm (Bryant, 1973). Mating occurs immediately and comparatively earlier at lower infection levels (Beckett and Pike, 1980). Eggs appear in the faeces of the susceptible host by 9-10 days from the time of ingestion.

Cross, Dawson, Scott and MacDonald (1958) noted that the egg production peaked by 16-18 days after infection and fluctuated about this level for four to five weeks then decreased steadily until it ceased about eight months after infection, with no female worms remaining at autopsy. Each female is capable of producing up to 31,000 eggs during its lifespan (Keymer and Hiorns, 1986a).

Although the normal predilection site for this parasite is the anterior part of the duodenum (Bawden, 1969), a number of factors have been observed to influence the position the worms occupy. At four to six days after infection, worms occupy about 50% of the small intestine but as infection progresses, the worm population became aggregated towards the anterior section of the small intestine (Lewis and Bryant, 1976). Dobson (1961) reported similar migration of the adult to a more anterior position and suggested that the movement was stimulated by the secretion of bile into the duodenum, with the worms tending to move to a position anterior to the entry of the bile duct. Bile or its constituents are known to influence activation and exsheathment or excystation of several helminths (Hwang, 1960; Campbell, 1963a; Dawes and Hughes 1964; Mapes, 1972; Caley, 1974). Sukhdeo and Croll (1981) in a detailed experiment also

presented data to show that bile contributes to the exsheathment and distribution of the larvae of *H. polygyrus* in the intestine.

Oxygen tension, pH and the supply of dietary nutrients have also been suggested to influence the anterior movement of adult *H. polygyrus* (Bawden, 1969).

2.1.3 Pathogenicity and Pathology

Local effects of gastrointestinal parasites on their host vary from species to species and may include one or a combination of the following: traumatic damage, inflammation, granuloma formation, altered villus architecture, altered smooth muscle architecture, mechanical obstruction, intestinal enzyme deficiency, impaired absorption, altered responses to hormones, altered motility, and competition for nutrition with the host (review by Castro, 1990).

2.1.3.1 Gastrointestinal changes

H. polygyrus is generally considered not to be very pathogenic in outbred mice. A primary infection is chronic, lasting up to eight months. Spurlock (1943) observed that the signs of severe or near fatal infection included progressive emaciation, sensitivity to cold, roughness of coat and reddish-brown to watery diarrhoea but terminally, the faeces formed a large anal plug at death. Much of the intestinal pathology is associated with the short histotropic larval phase in the mucosa, which is characterised by a strong inflammatory response in the gut walls. Liu (1965a) described a local gastritis, with lesions similar to that caused by *Ostertagia* spp., in the fundic region of the stomach four hours after oral infection. According to Jones and Rubin (1974), histopathological changes in the form of discrete foci of cellular reactions containing eosinophils, lymphocytes and macrophages are observable in the small intestine of immunized mice from one to three days after challenge infection.

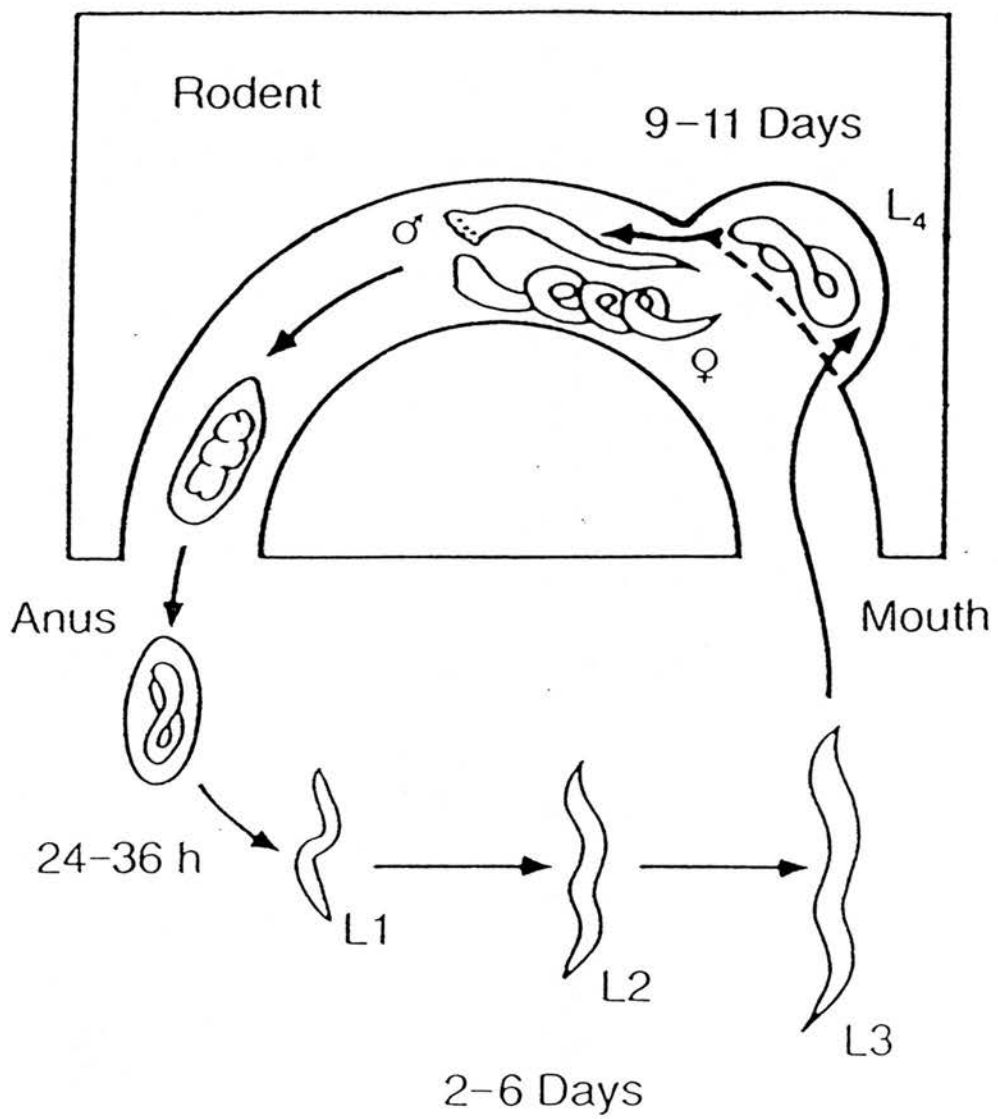

FIGURE 2.1 Diagrammatic representation of the life cycle of *H. polygyrus* (after Monroy and Enriquez, 1992)

During invasion of the intestinal tissue, the larvae are believed to release necrotizing substances which cause local tissue destruction and inflammation of the mucosa and submucosa with thrombi in damaged blood vessels (Liu, 1965a). The larval phase provokes the most dramatic pathological changes at the site of encystment in the muscularis externa which result in the formation of granulomata or parasitic nodules similar to those produced by various species of *Oesophagostomum* in man, pigs and ruminants (Thomas, 1910; Schwartz, 1931; Spindler, 1933; Fourie, 1936 and Andrews, 1942). The final emergence of the juveniles results in a catarrhal enteritis due to a transient inflammation. As a result of the intestinal trauma, causing petichial and ecchymotic haemorrhage, blood clots may be seen in the small intestines of infected animals (Spurlock, 1943; Liu, 1965a). Macroscopically, the infected intestine is enlarged and translucent.

The few available studies of the cellular changes that occur in the gut mucosa during infection with *H. polygyrus* indicate that, as with other intestinal parasites, there is an increase in the intraepithelial lymphocytes, globular leucocytes, and goblet cells, especially in the resistant mice (Monroy and Enriquez, 1992). Liu (1965b) reported the presence of abnormal numbers of polymorphonuclear, plasma cells and eosinophils in the intestinal mucosa of infected Webster mice. These changes were associated with oedema of the mucosa and dilation of numerous lymphatic vessels. Mast cell hyperplasia which is seen in infection by helminths such as *Nippostrongylus brasiliensis, Strongyloides ratti, Trichostrongylus colubriformis, Trichinella spiralis* and *Taenia taeniaeformis* is totally absent in *H. polygyrus* infection.

Pathogenicity of enteric parasites depends predominantly on direct contact with the mucosa (Castro, 1990) and the pathology of enteric nematodes that feed directly on the host tissue can be very profound (Soulsby, 1982). These infections are associated with inflammatory changes such as cellular infiltration into the mucosa, mucosal oedema with leakage of plasma into the lumen of the intestine, villous atrophy, mucus hypersecretion, disruption of epithelial integrity and

increased gut motility (Moqbel and MacDonald, 1990). In contrast, *H. polygyrus* suppresses the inflammatory responses (Behnke, Cabaj and Wakelin, 1992) that form the basis of many pathological changes. However, in very heavy infections perforation of the anterior duodenum and peritonitis have been reported (Spurlock, 1943).

Little is known about the pathophysiology of *H. polygyrus* infection but since its pathology is similar to that of the lumen dwelling trichostrongyles of ruminants, it is likely that such effects as alteration of intestinal motility, digestion and absorption (Holmes, 1987; Steel, Jones and Wagland, 1990) will also accompany heligmosomoidosis in mice.

2.1.3.2 Changes in lymphoid organs

H. polygyrus infection has been observed to result in pathological changes in other organs of the body apart from the small intestine. Remarkable colour, size and structural changes have been noted in the haemopoietic and lymphoid systems, notably, in the mesenteric lymph glands, the spleen and the liver. (Spurlock, 1943; Price and Turner, 1983; Ali and Behnke, 1985; Parker and Inchley, 1990a and b). However, while *N. brasiliensis* and *Trichuris muris* infection in C57BL mice produced thymic atrophy, probably due to the release of non-specific cytotoxic substances, *H. polygyrus* infection failed to influence the thymus weight even when administered intravenously (Price and Turner, 1983).

The secondary lymphoid organs especially the spleen are enlarged by 30-90% in mice infected with *H. polygyrus*. In most strains of mice, splenomegaly starts within three days of infection and may last for at least two to four weeks (Price and Turner, 1983; Losson, Lloyd and Soulsby, 1985). Although Baker (1955) suggested that anaemia or some 'unidentified factor' was responsible for splenomegaly in infected mice, recent studies have shown that the rate of increase in the size of these organs is responder-status dependent (Ali and Behnke, 1985) and also roughly proportional to the intestinal worm burden. If however the worm burden is terminated by anthelmintic treatment, the enlarged organs rapidly regress

to their original state. This finding led Ali and Behnke, (1985) to conclude that the enlargement of these organs may be due to the release of toxins and or antigens from the adult worms in the intestine (Parker and Inchley, 1990b). The actual increase in organ size has been shown to be due to an increase in the cellularity, especially in the numbers of lymphocytes and reticuloendothelial cells and red blood cells in the case of the spleen (Liu, 1965b). This has been confirmed by the recent studies of Parker and Inchley (1990a) in which cell division and enlargement were monitored within the spleen and the mesenteric lymph nodes of infected mice by the rate of ^{125}I-5-iodo-2'-deoxyuridine incorporation.

2.1.3.3 Haematological changes

Initial stages of primary infection with *H. polygyrus* is associated with pronounced qualitative and quantitative haematological changes (Baker, 1962; Cypess 1972; Ali, Behnke and Manager, 1985). The emergence of the juveniles coincides with a marked reduction in the packed cell volume (PCV) and the haemoglobin level but these return to preinfection levels about 27 days after infection (Baker, 1955). In primary infections, a leucocytosis comprising of relative neutrophilia and lymphocytosis begins as early as three days after infection. The leucocytosis peaks between seven and nine days after infection, corresponding to the period of emergence of the juveniles from the the mucosal wall (Baker, 1962). In addition, five to six days after infection Prowse, Ey and Jenkin (1978) observed a significant increase in the monocyte count at peak leucocytosis. Although Baker (1962) did not record a striking eosinophilia, other studies (Cypess, 1972; Ali *et al.*, 1985) demonstrated a distinct but transient eosinophilia in animals undergoing primary and secondary *H. polygyrus* infections. Thus the population of all categories of white blood cells (WBC) are affected by *H. polygyrus* infection in mice.

2.1.3.4 Factors affecting pathology and pathogenesis

Numerous factors have been identified which influence the pathogenesis of gastro-intestinal helminth infection. From the large volume of literature on this

subject, the salient factors appear to be the pathogenic mechanisms of the parasite, the level and duration of infection, the genetic background of the host, the plane of nutrition, the unique characteristics of the injured tissue or organ, the age of the animal and the immunological status of the host (reviewed by Holmes, 1987 and Castro, 1990).

Where the functional and physiological capacity of both ruminants and rodents have been examined during gastro-intestinal nematode infection, the severity of the pathogenic effects has been found to relate to the level of infection (Castro, Olson and Baker, 1967; Taylor and Pearson, 1979; Steel *et al.*, 1980; Sukhdeo and Mettrick, 1984).

The nutritional status of rodents has been extensively studied and has been found to affect the course of infection of enteric nematodes (Slater and Keymer, 1986a,b). Its impact is through its modulating effect on the immunocompetence of the host, which may be shown as an inability to control infection or to respond to vaccination (Wakelin, 1989). Malnourished hosts show significantly depressed immune responses to infection and the nematode parasites they harbour survive longer (Abbot, Parkins and Holmes, 1985; Cummins, Duncombe, Bolin, Davis and Yong, 1987; Slater, 1988; Slater and Keymer, 1988; Mansour, Rowan, Dixon and Carter, 1991). Under field conditions, malnutrition has also been found to exacerbate gastrointestinal parasitism in ruminants (Schillhorn van veen, 1974; 1978; Holmes, 1991). Although the actual mechanisms of immunodepression by malnutrition is not yet fully understood, reduction in the number of circulating T-cells and and an increase in the suppressor T-cell activity may be contributing factors (Wing, Magee and Barczynski, 1988).

2.1.4 Immunological Considerations

The pathological outcome of gastro-intestinal helminth infection depends on the successful establishment, development and survival of the parasite in the host. This is however determined by an interplay of two principal factors namely, the immune status of the host and the ability of the parasite to evade the host's

immunological responses. The chronic nature of many gastro-intestinal helminth infections in humans and in domestic animals has been explained in terms of the host and the parasite's derived host immunomodulation, resulting in unresponsiveness which tilts the balance in the host-parasite relationship in favour of the parasite (reviewed by Behnke, 1987).

2.1.4.1 Factors influencing host immunity

Several factors have been shown to have a profound effect on the immune status of the host with regard to its immune responses against gastro-intestinal helminths. These include drug intervention, naturally arising weaknesses and host derived influences.

In experimental infections, drugs such as dexamethasone (a corticosteriod which is directly cytotoxic to monocytes and immature lymphocytes), cyclophosphamide (a cytotoxic drug) and cimetidine (a histamine H2 inhibitor) have been used to induce immunosuppression in animals through their action on the cells of the immune system (Wakelin, 1970; Wakelin and Selby, 1974; Adams, 1988, Presson, Gray and Burgess 1988; Adams, 1990; Newlands, Miller and Jackson, 1990). In the case of *H. polygyrus*, treatment of resistant host with cortisone has been reported to result in the reactivation of development of larvae that were arrested in the intestinal wall (Behnke and Parish, 1979b).

In addition to protein malnutrition, which reduces the effectiveness of immunization of mice against *H. polygyrus* (Slater and Keymer, 1988), other factors associated with age and lactation are also known to enhance the pathogenicity of gastro-intestinal nematodes by depressing the protective responses. For instance, delay or failure of the normal expulsion phase has been observed in young or lactating rodents carrying *N. brasiliensis* (Ogilvie and Love, 1974; Connan, 1973). Connan (1972) also demonstrated that lactation interfered with the expression of immunity to secondary infection in rats that had been previously immunised against *N. brasiliensis*. These findings concur with field observations in ruminants where an increase in the faecal egg counts, referred to as

the periparturient rise, has been associated with a reduction of immune status of the host due to lactogenic endocrine changes accompanying parturition (Connan, 1968; Agyei, Sapong and Probert, 1991).

The role of the endocrine hormones in sex-linked resistance is not clear because there are conflicting reports. Van Zandt, Cypess and Zidian (1973) and Bryant (1974) did not see any sex-linked resistance of mice to primary *H. polygyrus* infection, but Dobson, (1961, 1962) and Bawden (1969) considered that a higher resistance in females could be explained in terms of an oestrogenic effect while Newton, Weinstein and Sawyer (1962) attributed this effect to the influence of intestinal flora. These variations may be host-strain dependent.

An additional complexity in the operation of the sex-linked responses is the possible influence of maternal infection on the immune responsiveness of their progeny. According to Lloyd and Soulsby (1987), lambs born to infected ewes developed specific antigen-reactive lymphocytes earlier than their mates from worm free ewes, but protective resistance was negatively correlated with lymphocyte responsiveness. This may be attributed to putative suppressor activity. The precise mechanisms involved in these phenomena are as yet poorly understood but it is thought that this temporary period of neonatal immunosuppression safeguards the suckling young against becoming sensitized to proteins in the mother's milk (Behnke, 1990).

Host genotype plays a fundamental role in the pathogenesis of an infection through its influence on the initiation, regulation and expression of the immune response (Wakelin, 1989). Genetic control may be mediated via innate host characteristics such as physical barriers to be crossed by the infective agent. For example in schistosomes, hookworms and *Strongyloides* spp, where the infective stage actively penetrates the skin of the host, susceptibility to infection could be influenced by the different biochemical characteristics of the skin and the acellular connective tissue constituents, which can affect the efficacy of larval enzymic secretion (Lewert and Lee, 1957; Lewert and Mandlowitz, 1963). In addition,

genetic control can be mediated through acquired host characteristics. The changes which result from an exposure to infection may increase susceptibility or render the host resistant to further infection. These changes may be physical, for example, the thickening of epithelial structures after helminth penetration, or direct modulation of the immune response through an immunologically based anti-parasite activity (reviewed by Wakelin, 1978a).

Laboratory animals, especially the rodents - both inbred and out-bred, have been reported to vary in their responses to similar infections. One very good example is that of *T. muris* infections in mice, where there is variation in the rate of worm expulsion between strains of inbred host and also between individuals of out-bred strains (Worley, Meisenhelder, Sheffield and Thompson, 1962; Campbell, 1963b; Wakelin, 1975a,b). The available information suggests that a variability in the production of the antibody response could account for the wide range of strain differences at the time of expulsion (Wakelin, 1975b). Through selective breeding of Schofield mice, it was possible to demonstrate that responsiveness to *T. muris* was inherited as a dominant characteristic (Wakelin, 1975c). Both Major Histocompactibility Complex (MHC)-linked and non-MHC genes are said to be associated with the host's capacity to expel parasites (Wassom, Wakelin, Brooks, Krco and David, 1984; Wakelin and Donachie, 1983) but the strongest influence is associated with non-MHC genes (Wakelin, 1980, 1986)

Unlike *T. muris* and *T. spiralis*, *H. polygyrus* does not evoke spontaneous expulsion during primary infection in mice. Genetic variation in the response of out-bred and inbred strains to primary infection (Spurlock, 1943; Liu, 1966) or following challenge after an immunization regime (Cypess and Zidian, 1975) have been investigated. Based on the evaluation of survival of the host, worm burden, sex ratio and the faecal egg output as well as on the antibody response, commonly used laboratory strains have been categorize as slow (low or weak), moderate or fast (high or strong) responders (Cypess, Lucia, Zidian and Rivera-Ortiz, 1977; Prowse and Mitchell, 1980; Mitchell, Anders, Brown, Handman, Robert-

Thompson, Chapman, Forsyth, Kahl and Cruise, 1982; Jacobson, Brooks and Cypess, 1982; Wahid, Robinson and Behnke, 1989b). Thus the three possible outcomes of infection may be total rejection (as in the SJL, LAF1/J and N2B strains); "adoptive tolerance" with little effect on the host and a low worm burden (as in BALB/c mice) or defective resistance resulting in long lasting infection with high worm burden (as in C57BL/10 mice) (Mitchell and Prowse, 1979).

It is postulated that the course and the outcome of primary infection with *H. polygyrus* is influenced by multiple locus host genes (Wahid *et al.*, 1989b). It is possible that gene products of at least two foci within the H-2 haplotype are crucial in determining the response phenotype of mice to primary *H. polygyrus* infection. The results of the work of Behnke and Wahid (1991), who showed that there was no significant difference in worm survival during primary infection between B10 congenic hybrid strains of mice which were either I-E^{+ve} or I-E^{-ve}, however, does not fit in with the hypothesis of Wassom, Krco and David (1987) who suggested that the expression of MHC II I-E gene products correlates with susceptibility to *T. spiralis* and secondary *H. polygyrus* infection.

2.1.4.2. Mechanisms of immunity against H. polygyrus

2.1.4.2.1 The role of humoral immunity

Several mechanisms for immunity against *H. polygyrus* have been proposed. Panter (1969a) suspected, although without much convincing evidence, that actions of antibody were directed against incoming larvae. It has also been proposed, that in immune mice, a fresh intake of larvae initiated an anaphylactic reaction and that the changes associated with this reaction prevented a large proportion of the invading larvae from becoming established (Panter, 1969b; Jones and Rubin, 1974).

As with *N. brasiliensis* in immune rats, the physical changes associated with anaphylaxis might facilitate the passage of anti-worm antibodies into the subepithelial spaces and intestinal lumen (Barth, Jarrett and Urquhart, 1966).

Crandall, Crandall and Franco (1974) assessed by fluorescent antibody technique the local immunoglobulin (Ig) levels and the antibody titres of these Ig classes at intervals following initial and challenge *H. polygyrus* infections of mice. IgG_1 which, paralleled increase in the serum Ig concentration was the main Ig class that responded to infection (within two weeks). The local IgA concentration of the small intestine did not differ significantly from the preinfection state but the cell infiltrates in the mucosa and submucosa of immunized animals contained numerous Ig-containing cells.

No crucial role for the intestinal antibody responses has yet been defined, but this does not rule out a role for antibody because they could augment the local anaphylactic reaction (Crandall *et al.*, 1974) or interfere with the worms ability to feed (Smith, 1988). Various other functions of antibodies such as biological inactivation (i.e. toxin neutralization or enzyme inactivation), agglutination of particulate antigen, opsonisation to facilitate phagocytosis and the release of lysozomal enzymes as in antibody dependent cell mediated cytotoxicity (ADCC) (Kassis, Aikawa and Mahmoud, 1979; Capron, Dessaint, Capron, Joseph and Pestel, 1980) have been suggested (reviewed by Wakelin, 1984a).

During both primary and secondary *H. polygyrus* infection, the pattern of serum immunoglobulin isotypes, particularly of IgG subclasses, is altered (Molinari, Ebersole and Cypess, 1978; Pritchard, Williams *et al.*, 1983; Williams and Behnke, 1983). In primary infections the serum IgG_1 levels rise within two weeks to a level twice that in the controls (Crandall *et al.*, 1974; Molinari *et al.*, 1978) and in challenge infections, the IgG_1 level rises even more considerably (Prowse *et al.*, 1978). In immune transfer studies (Behnke and Parish, 1979a), the appearance of protective antibodies coincides with the rise in IgG_1 level, suggesting that this Ig subclass might have a protective role in the immune serum. Pritchard *et al.* (1983) observed that immune sera raised in CFLP mice by repeated infection contained 24 mg/ml IgG_1 compared with a resting level of 2.4mg/ml. Absorption studies revealed that 48% of the purified IgG1 from the immune serum

reacted with adult *H. polygyrus* antigen and it was only this isotype that caused a significant reduction in worm numbers as well as severe stunting of the worms. Urban, Katona, Paul and Finkelman (1991b) have also recently reported of polyclonal IgE response in *H. polygyrus* infections.

2.1.4.2.2 The role of cell-mediated immunity

Thymus derived cells are also believed to be important in protective immunity to helminths because they regulate some effector elements associated with helminthosis such as local mastocytosis, eosinophilia and IgE production (Jarrett and Bazin, 1974; Butterworth, 1977; Urban, Katona, Dean and Finkelman, 1984). The role of T cells in immunity to *H. polygyrus* infection was investigated by Bartlett and Ball (1974) who compared worm establishment in groups of mice that were thymectomized with those of intact animals. Mice depleted of thymus derived cells formed no inflammatory nodules and were unable to delay maturation of larvae. Cell mediated immunity was confirmed by the observation that *in vitro* migration of leucocytes obtained from resistant animals was inhibited in the presence of antigen derived from whole adult worm, whereas leucocytes from uninfected mice migrated normally. From all these studies it may be concluded that T-lymphocytes are necessary for the inflammatory response to larvae present in the tissues which also delays the maturation and elicits encapsulation and death of the larvae in immune animals.

Other studies employing athymic nude (nu/nu) mice, thymic-derived cells, *in-vitro* interleukin 2 (IL-2), gut associated lymphoid tissue cell secretions or *in vivo* depletion of $CD4^+$ T-cells indicate that helper T (T_H) cells have an important role in host immune response to *H. polygyrus* infection (reviewed by Monroy and Enriquez, 1992). Urban, Katona, Paul and Finkelman (1991a) recently examined the role of $CD4^+$ and $CD8^+$ cells in protective responses to *H. polygyrus* infection by selectively depleting, *in vivo*, either T-cell subpopulation with rat monoclonal antibody specific for the appropriate determinants. Their results indicated that mice which had received anti-CD4 treatment during primary infection showed

higher faecal worm count. The almost complete protection obtained in immunized BALB/c mice was also abrogated by this treatment. In addition, the anamnestic serum IgE response to a challenge infection was reduced over 80%. Since anti-$CD8^+$ did not produce any appreciable effect, it was concluded that $CD4^+$ T cells regulate the host's protective immunity, worm fecundity and IgE levels in *H. polygyrus* infection. Treatment of mice with anti-CD4 antibody prior to and during a primary infection with *N. brasiliensis* larvae also prevented the typical spontaneous expulsion of adults and blocked mucosal mastocytosis and IgE production (Katona, Urban, and Finkelman, 1988). However, anti-CD4 antibody had no effect on the protective response to challenge infection with *N. brasiliensis*, suggesting that the effector mechanisms that affect parasitic nematode survival in the host may not always be $CD4^+$ cell dependent.

The precise mechanism of $CD4^+$ cell regulation of protection is not yet known, although depletion of $CD4^+$ cells from animals leads to elimination of the T_H1 and T_H2 subpopulation which produce lymphokines that have been implicated in protective responses to helminthosis (Finkelman, Pearce, Urban and Sher, 1991; Scott and Kaufmann, 1991). It is suggested that *H. polygyrus* induces T_H2 cell responses and that immunity to challenge is IL-4 dependent but may not be IgE dependent (Else and Grencis, 1991; Scott and Kaufmann, 1991; Monroy and Enriquez, 1992).

2.1.4.2.3 The role of non-lymphoid cells

Apart from the T-cells and antibodies, non-lymphoid effector cells have also been associated with protective responses in helminth infections. Their involvement is usually shown by marked intestinal inflammation which is reflected both in the cellular changes in the intestinal mucosa and in the altered physiochemical condition of the gastrointestinal tract. Available information suggests that inflammation has a protective role and is a consequence of an immune response to parasites (reviewed by Wakelin, 1978b; Moqbel and MacDonald, 1990). Neutrophilia and eosinophilia has been directly associated

with intestinal inflammatory responses to tissue larvae. Although neutrophils and eosinophils from immune mice exhibit larvicidal activity in the presence of inactivated serum, none of these cells nor serum alone kill the larvae of *H. polygyrus* (Monroy and Enriquez, 1992). If eosinophils need protective antibody in order to mediate in ADCC as they do in schistosomosis (Kassis *et al.*, 1979; Capron *et al.*, 1980), then the the lack of protection characteristic of primary *H. polygyrus* infection may be explained by a lack of parasite specific IgG_1.

In the *T. spiralis*-mouse model, the precise characteristics of the inflammatory responses and the mechanisms involved in its generation and in the subsequent expulsion of parasite have been reviewed by Wakelin and Denham (1983). The inflammatory cellular infiltration which accompanies worm invasion is associated mainly with mast cells, eosinophils, villous atrophy, increased gut motility, increased levels of inflammatory mediators and high goblet cell activity, which lead to mucus secretion (Moqbel and MacDonald, 1990). These changes almost certainly contribute to the removal of worms from the gut.

Although the generation of immune response is mainly elicited by a particular parasite stage, the expression of response may be quite non-specific in that adult worms of homologous and heterologous species present may be equally expelled (Dineen, Gregg, Windon, Donald and Kelly, 1977; Behnke, Bland and Wakelin, 1977; Bruce and Wakelin, 1977; Ferretti, Gabriele, Palmas and Wakelin, 1984). The relative importance of individual components involved in worm expulsion vary between host parasite systems but the overall pattern seems to be similar (Wakelin, 1986). *H. polygyrus* especially in primary infection is however particularly resistant to non-specific components of the intestinal rejection process (Day, Howard, Prowse, Chapman and Mitchell, 1979; Behnke, *et al.*, 1992).

2.1.4.3 Evasion of host immunity

As discussed earlier, primary *H. polygyrus* infection is not readily affected by responses, so an initial infection lasts up to eight months in most strains of mice.

Both in *H. polygyrus* and in other chronic helminth infections, it is believed that the parasites actively evade their hosts' immune responses by some immunomodulatory process (reviewed by Barriga, 1984; Behnke, 1987). It is known that even if primary infections of *H. polygyrus* are terminated at the larval stage by anthelmintic treatment, protection is already secured against secondary infection (Behnke and Robinson, 1985), but infections allowed to proceed to adulthood produce non-specific immunodepression in immunocompetent hosts (Ali and Behnke, 1984). Also, adults transplanted by laparotomy into recipient mice negate the immunizing effect of L_4 stages and immune serum (Behnke and Parish, 1979a; Behnke, Hannah and Pritchard, 1983; Behnke and Robinson, 1985). These experiments have led to the conclusion that it is the adult stage that has the greater capacity to modulate the host's protective responses to its own advantage.

Several mechanisms have been suggested which might permit the chronic nature of primary *H. polygyrus* infections, but the most plausible one is the possibility of the nematode secreting locally-acting immunomodulatory factors which affect both the induction and the expression of the homologous immune response (Behnke *et al.*, 1983; Pritchard and Behnke, 1985). Very recent experiments (Behnke, Cabaj and Wakelin, 1992) suggest that in addition to the immunomodulatory strategy employed by adult *H. polygyrus* to prevent the intestinal responses against them, the worms have a second line of defence in their resilience to those responses which they were unable to prevent. Thus they escape immune expulsion by residing in "safe havens" created by their activity, which result in a local nonspecific immunodepression (Behnke, 1987), and through their tolerance of hostile environments.

The mode of operation of this immunomodulatory activity of the adult parasite has been a matter of speculation (Behnke, 1990). Since high concentration of serum IgG_1 observed during primary infection did not result in worm loss from the host (Crandall *et al.*, 1974; William and Behnke, 1983), it was proposed that the greatly elevated levels of serum IgG_1 may act to protect the parasite by

blocking potentially useful host responses (Bartlett and Ball, 1974; Dobson and Cayzer, 1982). Price and Turner (1986) observed that crude saline extracts of adult *H. polygyrus* administered with an intraperitonial immunization by ovalbumin in aluminium hydroxide depressed both the primary and the secondary IgG responses, delayed-type hypersensitivity and the *in vitro* splenic lymphoproliferative responses to ovalbumin. They suggested that the extract blocks the inductive phase of the immune response so that clonal expansion of primary antibody-producing cells, memory cells and T-lymphocytes is impaired.

Attempts are now being made to characterize the immunosuppressive proteins produced by adult *H. polygyrus* so that an understanding of their biochemistry, synthesis and mode of immunological activity may enhance successful preparation of a vaccine. Although excretory/secretory (E/S) protein antigens resolved between 14 and 20 kilo Daltons(kDa) on sodium dodecyl sulphate polyacrylamide gel-electrophoresis (SDS-PAGE) had earlier been associated with immunoregulatory activity (Monroy, Dobson and Adams, 1989), Pritchard, Lawrence, Appleby, Gibb and Glover (1992) have very recently identified additional proteins at 67 and 54 kDa produced by 10 to 20 day old worms maintained in culture for 24 hours. The immunomodulatory factor(s) were intrinsically stable at pH2, room temperature and 50°C but were destroyed by boiling. The nature and the mode of action of these factors are still not clear. For the host to gain control of the parasite, 'neutralizing' immune responses must be directed against the immunomodulator.

2.2 *TRYPANOSOMA (NANNOMONAS) CONGOLENSE*

T. congolense is one of the Salivarian species of trypanosomes of the subgenus, *Nannomonas*. Morphologically, it is smaller than the other mammalian trypanosomes, measuring 11.2-13.8μm. The kinetoplast of this flagellate is typically marginal and without a free flagellum.

2.2.1 Life Cycle

The life cycle of this protozoan involves alternation in an invertebrate intermediate host and the final mammalian host. The bloodstream forms (trypomastigotes) are taken up in a blood meal by various species of *Glossina* (tsetse-fly), in which they undergo cyclical development in the gut through an epimastigote stage to the infective metacyclic forms (metatrypanosomes). Details of this development have been described by many authors including Hoare (1972) and Soulsby (1982). The infective metacyclics, which are usually located in the mouth parts (proboscis) of the insect-host, are inoculated into the mammalian host during feeding. Once in the bloodstream, the parasites are transformed into trypomastigotes which replicate by binary division. The prepatent period which varies according to the host, ranges from one to three weeks following an infected bite and four to five days when the transmission is by mechanical blood inoculation. Unlike *T. brucei*, which also occur in tissues and organs, *T. congolense* bloodstream forms reside strictly in the vascular system, especially in the capillaries where they may be very abundant (Hoare, 1972).

2.2.2 Pathology and Pathogenesis

All species of domestic mammals are susceptible to infection with *T. congolense* but vary in the severity of the disease they sustain (Kramer, 1966; Killick-Kendrick and Godfrey, 1963; Baldry, 1964; Hoare, 1970, 1972; Agu and Bajeh, 1986; Agu, Kalejaiye and Olatunde, 1989; Omeke and Ugwu, 1991). *T. congolense* infections may occur singly or in conjuction with other species such as *T. vivax* or *T. brucei.*

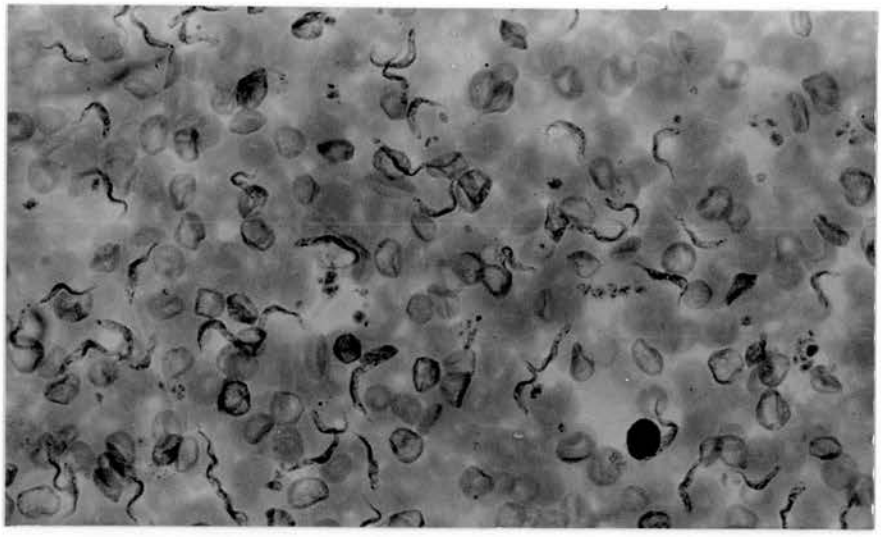

PLATE 2.3 *Trypanosoma congolense* in a blood smear of mouse (Giemsa stain)

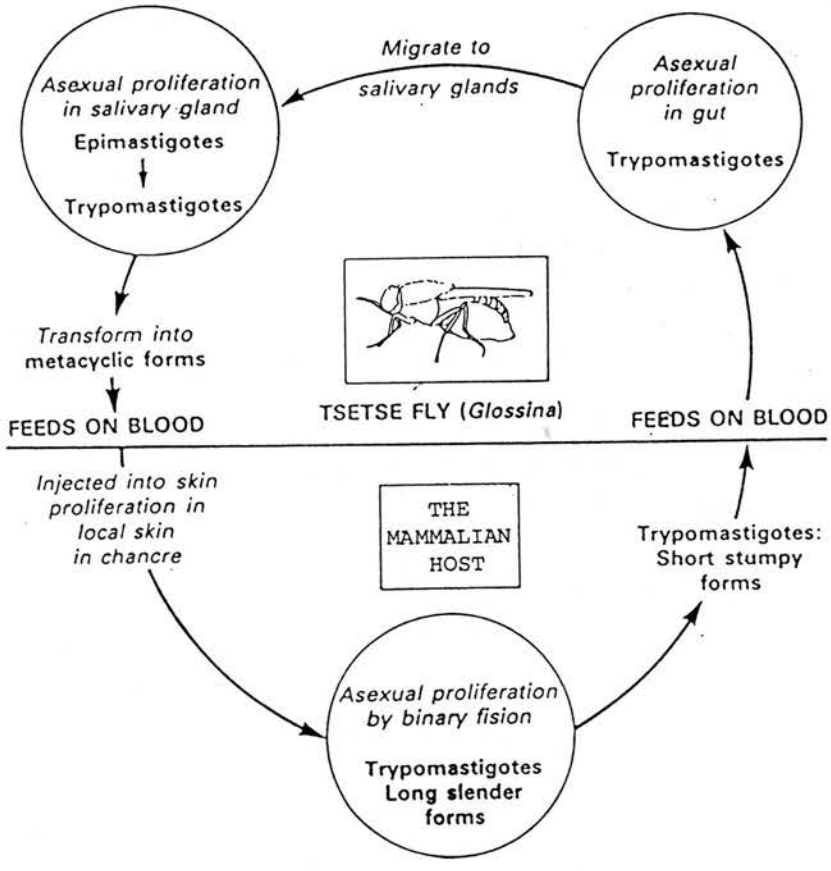

FIGURE 2.2 Diagrammatic representation of the life cycle of *T. congolense* (after Maizels, 1990)

Rapidly dividing trypanosomes cause debilitation in mammalian hosts both by consumption of tissue fluid components and by release of toxic secretions which interfere with normal physiology (Hoare, 1972). The harmful effects may thus result in overt disease which is generally characterised by severe anaemia, weight loss, cachexia, reproductive disorders, general loss of productivity and the eventual death of untreated animals (Losos and Ikede 1972; Ikede, Elhassan and Akpavie, 1988).

The pathology of *T. congolense* in domestic and laboratory animals has been reviewed by Losos and Ikede (1972). In laboratory rodents such as mice and rats, the infection is characterised by a progressively increasing parasitaemia but may run a chronic course in guinea pigs, rabbits and in ruminants under field situations. One consistent pathological feature of trypanosomosis is anaemia (Anosa, 1988) and this, as assessed by PCV, has been recognised as a good indicator of the severity of the disease (Feron, d'Ieteren, Itty, Maehl, Mulungo, Nagda, Paling, Rarieya, Sheria, Thorpe and Trail, 1988). Persistent anaemia and congestive heart failure due to myocardial damage may be a major cause of death in trypanosome-infected animals (Murray, Murray, Jennings, Fisher and Urquhart, 1974; Gardiner, 1989). The aetiology of this anaemia is complex but the most important component appears to be haemolysis, a conclusion based on the observation of a reduction in the total mass and life span of the erythrocytes and the occurrence of erythrophagocytosis, haemosiderosis and hyperbilirubinaemia in some infected animals (Anosa, 1983a). In *T. vivax* infection, the haemolysis has been attributed to the action of parasite-associated neuraminidase which causes cleavage of the sialic acid in the erythrocytes, rendering them more prone to phagocytosis (Esievo, Saror, Kolo and Eduvie, 1986). Other factors contributing to the anaemia include haemodilution due to increase in the plasma volume (Valli, Forsberg and McSherry, 1978) and direct haemorrhage from various organs, as observed in *T. vivax* infections (Anosa, 1983b).

Unlike the effects of *T. brucei* infection in rodents, which produce a considerable reticulocyte response to compensate for the loss of red blood cells (Jennings, Murray, Murray and Urquhart, 1974), ruminants infected with *T. vivax* or *T. congolense* showed little or no such response (Valli and Mills, 1980; Igbokwe and Anosa, 1989). This lack of an erythroid response is suggestive of some form of bone marrow depression. It is suggested that this dyserythropoiesis which results from depressed levels of plasma erythropoietin, endocrine imbalance or parasite-associated neuraminidase may be a significant contributor to anaemia in most salivarian trypanosomosis (reviewed by Igbokwe, 1989). Although the causes of the erythropoietic depression are yet to be fully elucidated, it appears reasonable to associate it with the direct presence of the parasite in the blood, since the PCV of animals treated against trypanosomes quickly return to normal.

Splenomegaly, which is another constant feature of trypanosomosis in rodents, is believed to be caused by congestion, intrasplenic haemorrhage as well as by the haemopoietic response which results in metaplasia and hyperplasia of the red pulp (Losos and Ikede, 1972).

2.2.3 Antigenic Variation

Antigenic variation which is a phenomenon observed in the African trypanosomes has being the subject of many detailed reviews (Gray and Luckins, 1976; Vickerman, 1978; Turner, 1982), and it provides the means by which the parasite successfully evades host immunity (Behnke, 1990; Pentreath, 1991). Trypanosomes are covered by a dense protein coat consisting of a single protein species known as the variant surface glycoprotein (VSG) (Cross, 1975). Trypanosome populations are antigenically heterogeneous, consisting mainly of one variant antigenic type (VAT) of the trypanosome (homotype) with minor populations of suppressed variants (heterotypes). During an infection, B cells are stimulated through T-cell dependent and independent pathways to produce specific VSG anti-trypanosome antibody against the homotype. When sufficient antibody to the surface coat of the main population has been produced, the particular

population expressing the VSG is eliminated. However, following this, small numbers of an heterologous VAT which has assumed a different VSG repopulate the blood and the process of elimination is again repeated, resulting in a fluctuating parasitaemia. Each parasitaemic peak is now known to be constituted mainly of one antigenic variant of the trypanosome (homotype) with minor populations of suppressed variants (heterotypes). The repertoire of of VATs seems inexhaustible as it is thought that genetic recombination among the trypanosome populations may also be possible (Steinert and Pays, 1986). Although VAT-specific IgG and IgM are produced against each variant, the switching of the VSG to variants not catered for by the host immune system results in the establishment of a persistent chronic infection such as those commonly observed in the field.

The remarkable ability of trypanosomes to undergo antigenic variation may well result in immunosuppression and may be responsible for the inability to develop an effective immunoprophylatic agent against these parasites (Nantulya, 1986).

2.2.4 Immunology of Infection

After an infected tsetse bite or following intradermal needle challenge, a local skin lesion called a chancre develops in which the parasites multiply prior to dissemination to the blood stream (Emery, Akol, Murray, Morrison and Moloo, 1980). Since immune animals develop a smaller chancre it was suggested that such animal may generate a superior or more rapid local or general immune response which acts to reduce the number of trypanosomes in the skin (Murray, Morrison and Whitelaw, 1982).

However, animals that are challenged by needle infection, bypassing the dermal barriers still show their innate differences in immune responses which correlate with their ability to control parasitaemia. This suggests that antibody rather than the cell mediated local skin reaction may be the most important mechanism in immunity to trypanosomes. This is supported by the fact that thymus-deficient (nude) and thymus-deprived mice can control an initial

parasitaemia as successfully as normal mice (Wakelin, 1984b). Several studies in both laboratory animals and cattle indicate that IgM is the most important class of Ig in controlling parasitaemia (Luckins, 1976, Musoke, Nantulya, Barbet, Kironde and McGuire, 1981).

Murray *et al.* (1982) reviewed some studies which showed that there was a marked increase of approximately four to ten fold in the level of total serum IgM and a slight increase in the IgG_2 isotype in resistant C57BL mice during and following peak parasitaemia in *T. congolense* infection. By contrast A/J mice, a very susceptible strain, did not show a similar increase. Instead there was massive increase of the serum IgG concentration, especially the IgG_2 which increased up to ten fold. A trypanolysis test showed that all C57BL mice contained a lytic antibody compared to just one third of the A/J mice. Agglutination, neutralization and immune clearance assays confirmed that antibodies specific for the infecting trypanosome were only found the C57BL. The humoral response, as measured by hepatic uptake of radiolabelled parasites indicated that significant numbers of trypanosomes were cleared from the circulation after eight days of infection (MacAskill, Holmes, Whitelaw, Jennings and Urquhart, 1983). Thus significant differences in the quality, quantity and specificity of antibody against trypanosomes may play a key role in the host's resistance.

2.2.4.1 Immunosuppression

African trypanosomosis is known to cause nonspecific depression of the immune responses of infected animals to a variety of heterologous antigens including those of bacterial and viral origin (Murray, Morrison, Emery, Akol, Masake and Moloo, 1980) and sheep RBC (Wakelin, 1984b). Several studies have revealed that this suppression closely follows the onset of parasitaemia (reviewed by Roelants, 1986) and depends on the strain of the parasite (Sacks, Selkirk, Ogilvie and Askonas, 1980).

Several proposals have been suggested to account for the immunosuppressive action by trypanosomes. Some workers have been able to

demonstrate that the parasites have direct effects on the bone marrow as shown by inhibition of bovine granulocyte/macrophage *in vitro* colony formation by sera from calves infected with *T. congolense* and *T. vivax* (Kaaya, Valli, Maxie and Losos, 1979). Mwangi, Munyua and Nyaga (1990) suggested that the immunosuppression to anthrax spore vaccine observed in goats infected with *T. congolense* was due to antigenic competition, judging from the negative correlation between the parasitaemia and the level of suppression. Other experiments conducted in mice and cattle infected with *T. congolense* and *T. vivax* suggest that the immunosuppression results indirectly from the severe depression of both the total haemolytic complement and C3 (Urquhart and Holmes, 1987; Rurangirwa, Tabel, Losos and Tizard, 1980; Tabel, Losos and Maxie, 1980).

It has also been suggested that trypanosomes have a mitogenic effect upon host cells which leads to polyclonal activation, resulting in exhaustion of the antigen-specific B cells (Terry, Hudson, Faghihi and May, 1980), which is believed to lead to a loss of response to mitogens and antigens as well as loss of immunological memory (Wakelin, 1984b).

Apart from the above, a catalogue of other events that have been observed in animal models during trypanosome infection include severe impairment in specific antibody induction, rapid suppression of T cell responses, generation of non-antigen-specific T-suppressor cells, activation of macrophages, mediator release and suppressive activity on immune response and bone marrow cellular changes (Askonas, 1984). These multiple changes in macrophages and lymphoid cells are also believed to make the host refractory to normal signals of T- or B-cell clonal expansion and maturation following antigenic challenge. Exactly how the parasites cause these effects remains a matter of speculation. Recent investigations have focused on the activities of macrophages. After the uptake of trypanosomes by the macrophages in the presence of antibodies, they become important target cells mediating immune dysfunction (Askonas, 1984; 1985). This may be manifested as an inability of the cells to present antigens properly to the T-cells

and probably by a general suppression of T cell activity (Pentreath, 1991). How this suppressive activity is mediated by the macrophage to the effector cells is still not clear.

In a recent review, Olsson, Bakhiet and Kristensson (1992), presented evidence which indicates that during infection, *T. brucei* releases a diffusible molecule which triggers $CD8^+$ T-cells to produce gamma-interferon (IFN-γ), a cytokine which provides a growth stimulus for the parasite and modulates events in the host's immune and nervous systems. Apart from its direct growth-stimulating effects on *T. brucei*, IFN-γ is also known to have dramatic activating effects on macrophages and may suppress T-cell proliferation, thus attention is currently being focused on the role of this cytokine and other secretory products of macrophages and T-cells in trypanosomosis (Onah, 1992).

From the attempts made so far which have led to the identification of some key factors in the pathogenesis of trypanosomosis, it can be concluded that immunosuppression in African trypanosomosis is multifactorial involving complex immunoregulatory mechanisms that are still poorly understood.

2.2.5 Epidemiology and Control

Several species of *Glossina* (tsetse fly), the natural vectors of some trypanosomes, have been identified (Onyiah, Naisa, Riordan and Gregory, 1983). The discontinuous and overlapping distribution of these vectors makes any meaningful calculations or demarcation of the infested areas difficult. Climatic as well as geographical factors affect the distribution of these flies. Although it has been generally believed and reported that the rainy season favours an increase in the tsetse population (Baldry, 1964), Madubunyi (1986) in his three year intensive study of the same location in Nsukka area of eastern Nigeria 20 years after Baldry showed a completely contrasting picture. Madubunyi observed an increase in the fly population during the dry season and a decline during the rains which coincided with the period of intensive agricultural activity. It is believed that the agricultural activities such as tillage increased pupal mortality as the top soil (the breeding

grounds of the tsetse fly) are turned over. Such changes in the ecology and epizootiology of *Glossina* spp. inhabiting intensively cropped peridomestic agroecosystems may be expected to be more common due to increasing urbanisation and the associated intensive use of the ever-decreasing agricultural lands. In addition, there is increasing evidence of infections in areas presumed to be tsetse free (Nawathe, Sinha, Abechi, 1988; Joshua and Shanthikutmar, 1989). With these changes in the epizootiology, trypanosomosis may no more be regarded essentially as a rainy season disease restricted to those parts traditionally referred as the tsetse fly areas.

The incidence of disease is theoretically expected to be a function of the prevalence and activity of the vectors, so the epizootiology of the tsetse fly has been used as a guide to the distribution and the risk of infection. However, it has not been possible to directly correlate the population dynamics of the fly with infections or infection rates in domestic animals, even when the vectors are shown to feed preferentially on a particular breed (Madubunyi, 1987).

Control measures for trypanosomosis have been either directed against the vectors (*Glossina*) or against the parasites. In the past vector control included large scale killing of game (reservoir) animals and bush clearing to destroy the tsetse breeding sites. Although these methods are fairly successful, they create unacceptable ecological problems (Jordan, 1992). Currently, most anti-tsetse measures rely on the use of insecticides and use of traps. Apart from the inevitable reinvasion of the tsetse from surrounding areas without any control scheme, the above measures are expensive, thus measures directed against the parasites which include chemotherapy and chemoprophylaxis are most widely used. This approach has most success in the short term in endemic areas (Urquhart, Armour, Duncan, Dunn and Jennings, 1987).

Attempts to control trypanosomosis have been faced with many problems. For example, eradication of the vectors cannot be realistically extended to the whole area at risk, while prophylactic drug treatment has led to field resistance

(Roelants, 1986). All these, together with the biological phenomenon of antigenic variation of the trypanosomes which defies the host's immunological defence and negates immunological control methods in practice (Nantulya, 1986), point to the fact that African trypanosomosis still remains a major problem to be grappled with in the endemic areas. Selective breeding from the resistant breeds for increased productivity, perhaps combined with judicious drug therapy remains an option to be fully explored.

2.2.5.1 Natural resistance to trypanosomes

Based on the field observation that certain breeds of cattle, sheep and goats can survive in endemic *Glossina* infested areas without succumbing to the disease, these breeds were described as "trypanotolerant". Animals such as the N'Dama and Muturu (*Bos taurus*) tolerate trypanosomes well and in many cases appear to suffer no ill effects from the infection. In contrast, the Zebu (*Bos indicus*) and exotic European breeds are known to be very susceptible to trypanosome infections. This subject has been well reviewed by Murray *et al.* (1982).

Experiments with *T. congolense* have shown that strains of mice differ markedly in their susceptibility to infection as judged by survival. One such experiment by Morrison, Roelants, Mayor-Withley and Murray (1978) showed that C57BL/6, C3H/He and A/J strains of mice showed least, moderate and high susceptibility respectively. Low susceptibility in mice has been shown to be a dominant trait which is under complex and multigenic control (Morrison and Murray, 1979). In cattle the high heritability of trypanotolerance is indicated by the large percentage of crosses that retain the tolerance (Stewart, 1951; Chandler, 1952; Letenneur, 1978).

Trypanotolerant animals appear to have a superior capacity to control parasitaemia, although the mechanisms responsible for this are still obscure. Roelants (1986) suggested that differences in susceptibility may lie in the capacity of resistant mice to produce a T-independent IgM antibody in response to small amounts of immunogen early enough in the course of infection to control

parasitaemia. Although trypanotolerance is believed to be immunological in nature, it is associated with, some as yet, undetermined factors. For example Whitelaw, MacAskill, Holmes, Jennings and Urquhart (1983) failed to obtain an enhanced immune response in susceptible CFLP mice with a variety of immunostimulants and passive immunization. Recent studies by Otesile, Lee and Tabel (1991), which demonstrated marked differences between the plasma levels of C3 in resistant and susceptible mice, suggest that genetic differences in the alternate complement pathway, which has been recognised as an important effector mechanism in natural resistance to infection, might affect the resistance to *T. congolense* infection.

Apart from inherited resistance, there are reports which suggest that in the field, resistance to trypanosomosis does occur in both resistant and susceptible breeds (Murray *et al.*, 1982) but is more readily acquired by the former (Desowitz, 1959; Roberts and Gray, 1973).

2.2.6 *In vitro* Culture System

The entire life cycle of *T. congolense* can now be reproduced in *in vitro* cultures derived from trypanosome infected *Glossina* sp. In the presence or absence of a supporting cell monolayer (Gray, Cunningham, Gardiner and Luckins, 1981; Gray, Ross, Taylor, Luckins, 1984; Gray, Ross, Taylor, Tetley and Luckins, 1985). Ross, Gray, Taylor and Luckins (1985) were also able to directly adapt the bloodstream forms of four cloned West African stocks of *T. congolense* to continuous culture in the presence of bovine aorta endothelial cell monolayers. By changing the culture conditions, the mammalian forms were transformed to procyclic trypanosomes. Established cultures consisted of epimastigote forms which grew as adherent layers on the culture flasks. These could be serially passaged in the absence of mammalian or insect cells resulting in continuous cultures from which metacyclics could be harvested within two to four weeks of establishment (Gray *et al.*, 1981, 1984). Cultures are successfully re-established

after cryopreservation at -196°C without any loss of ability to produce the infective organisms (Gray *et al.*, 1984).

The successful cultivation of infective trypanosomes seems to depend on the initial adhesion of the parasite to the surface of the flask during differentiation to the epimastigote stage, which transforms to infective metacyclic forms (Gray *et al.*, 1984). This was confirmed by Hendry and Vickerman (1988) who observed that although prevention of attachment did not inhibit epimastigote division, it did prevent differentiation to metacyclics.

The *in vitro* culture systems have contributed greatly to our understanding of various aspects of trypanosomosis. Being able to obtain trypanosomes at specific stages of their life cycle has aides the study the course of development *in vitro* compared to that *in vivo*. In addition, these cultures produce sufficiently large numbers of parasites for biochemical and immunological analysis (Ross, 1987; Prain and Ross, 1988; Luckins, Frame, Gray, Crowe and Ross, 1986). *In vitro* cultures are now being used to screen the efficacy of trypanocidal compounds against these parasites (Ross and Taylor, 1990). Epidemiological studies, such as those of Frame, Ross and Luckins (1990), which identified and enumerated the various serodemes (strains) present in 17 stocks isolated from eastern Zambia, were also based on *in vitro* cultures.

2.3 CONJOINT INFECTIONS WITH HETEROLOGOUS PARASITES

Although many laboratory studies have been devoted to various aspects of infection with single species, most hosts in natural populations are infected with a number of different parasites at the same time and interspecific interactions may be important in the epidemiology of infections (Quinnell and Keymer, 1990). Interaction between homologous and heterologous species has been reviewed by Holmes (1973) and Christensen *et al.* (1987). Because of the bewildering array of factors that may influence the outcome of parasite interactions, appropriately planned and controlled experimentation has been used in an attempt to elucidate the pathogenesis of various concurrent parasite infections in man and animals

(Christensen *et al.*, 1987). Concurrent infection with two or more parasite species may commonly result in interactions which may give rise to enhanced pathogenicity (synergism) or reduced pathogenicity (antagonism) of one or both parasites. However, in certain cases, the two parasite populations may develop independently of each other with the resultant pathology being a simple additive effect of the individual parasite populations.

2.3.1 Antagonistic Interactions

Competitive interaction has been reported in different species of helminths occupying similar predilection sites. Such interaction among intestinal parasites may result in a change of location (Holmes, 1973). This may be due to sheer mechanical interference or as result of non-specific factors induced by an immunological response to one of the parasites (Christensen *et al.*, 1987). For instance, cellular changes in the small intestine as a result of *Ascaris* sp. infection or the reaction to the release of the eggs of *Schistosoma mansoni* have been shown to impair the establishment of *Hymenolepis diminuta* in mice (Bindseil and Andreassen, 1981; Andreassen, Odaibo and Christensen, 1990). The inflammatory reaction initiated by *T. spiralis* has also been shown to be detrimental to the development of this particular tapeworm (Behnke *et al.*, 1977). Thus competitive interaction may result in an apparent interactive immunity (Behnke, 1990) if one species initiates the host response and subsequent effector components act non-specifically to the detriment of the heterologous species.

Some closely related species are known to show cross-immunity (Lee, Grencis and Wakelin, 1982). Such parasites may interfere immunologically with each other's presence through their possession of similar or identical antigens. However, functional phylogenic cross-reacting antigens have not been linked in all cases of antagonism (Monrad, Christensen, Nansen and Frandsen, 1981).

Some protozoans such as *Toxoplasma gondii* and *Trypanosoma cruzi* have been shown to induce significant resistance to *Schistosoma mansoni* establishment in mice (Mahmoud, Warren and Strickland, 1976; Kloetzel, Faleiros, and Mendes,

1971; Kloetzel, Faleiros, Mendes, Stanley and Aria, 1973). The immunosuppression associated with *T. gondii* has been suggested to suppress in mice the granulomatous reaction to *S. mansoni* eggs in concurrent infections (Mahmoud, Strickland and Warren, 1977). *T. gondii* has also been shown to result in reduced establishment and enhanced expulsion of *T. spiralis* in rodents (Copeland and Grove, 1979; Yusuf, Piekarski and Pelster, 1980). Such protozoan-induced resistance to helminths is thought to be the result of enhanced macrophage-mediated non-specific resistance (Christensen *et al.*, 1987).

Helminths such as *Fasciola hepatica, S. mansoni* and *H. polygyrus* have been reported to show antagonistic effects on *Babesia microti* in concurrent infections (Mzembe, Lloyd and Soulsby, 1984; Fagbemi, Christensen and Nansen, 1985a,b). Several studies reveal that *S. mansoni* and *T. spiralis* are capable of causing antagonistic effects on several protozoans (see review by Christensen *et al.*, 1987). Helminth induced suppression of blood protozoan infections may be mediated immunologically by non-specific factors involving macrophage activation and reticulocytopenia (Ngwenya, 1982; Mzembe *et al.*, 1984).

Most antagonistic interactions are beneficial to the host due to effects such as reduced survival, rapid expulsion, migration to less favourable site and reduced fecundity of the parasites (Christensen *et al.*, 1987).

2.3.2 Synergistic Interactions

As discussed earlier, certain protozoa and helminths evade their host's immunological response by causing immunosuppression which, in some cases, extends the duration of infection or increases the host's susceptibility to infection by heterologous species (Christensen *et al.*, 1987; Behnke, 1990; Hughes, 1991). Several helminths including *N. brasiliensis* (Jenkins, 1975), *T. muris* (Jenkins and Behnke, 1977), *Hymenolepis diminuta* (Hopkins, 1980) and *T. spiralis* (Behnke, Williams, Hannah and Pritchard, 1987) have been shown to have extended survival in mice concurrently infected with *H. polygyrus*. Some conjoint infections such as those by *T. colubriformis* and *O. circumcincta* in sheep, may produce far greater

effects than would be predicted by a simple summation of the effects of the individual infections (Steel, Jones and Symons, 1982).

Other interesting helminth-helminth interactions which result in synergistic pathology have been observed in the field. For example, in calves primed with mixed infection of *O. ostertagi* and *C. oncophora*, the establishment of superimposed *D. viviparus* increased by 191% (Kloosterman, Frankena and Ploeger, 1989), but priming with a single species of these gastrointestinal parasites did not affect the establishment of *D. viviparus* (Kloosterman, and Ploeger and Frankena, 1990). Why this should occur remains unexplained.

Although interactions between systemic protozoal parasites and intestinal helminths have not been studied extensively, there are a few reports which indicate that concurrent infections involving these types of parasite affect their pathogenicity. Heterologous synergistic effects of protozoans on helminths may be reflected in increased establishment of the helminths. *T. congolense* in goats and *T. brucei* in rats have been reported to increase the burden of *H. contortus* and *N. brasiliensis* respectively (Urquhart *et al.*, 1973; Griffin *et al.*, 1981a). *T. brucei* may also delay or block the expulsion of several helminths including *Echinostoma revolutum, N. brasiliensis, H. diminuta* and *Trichuris muris* (Urquhart *et al.*, 1973; Phillips *et al.*, 1974; Christensen, Fagbemi and Nansen, 1984; Fagbemi and Christensen, 1984). Interference with the development of resistance to heterologous challenge infection is another way by which protozoans enhance the effect of helminth infections as shown in concurrent infections of *T. brucei* and *E. revolutum* in mice (Christensen *et al.*, 1984). Coccidia are also capable of suppressing the immune mechanisms of the host, expressed as a delayed inflammatory response or suppression of eosinophil mobilization (Upton, Mayberry, Bristol, Favela and Sambrano, 1987).

Experimental evidence suggests that the synergistic interactive effects of these unrelated parasites which occupy different regions of the body is mediated through the immune system by one or a combination of mechanisms such as

suppression of eosinophilic and other cellular components of resistance to helminth, depressed or gross impaired production of local and systemic protective Igs (Urquhart *et al.*, 1973; Copeland and Grove, 1979; Wedrychowicz, Maclean and Holmes, 1984; Christensen, *et al.*, 1987).

Concurrent infections with intestinal protozoa of *Eimeria* spp and various nematodes of ruminants and rodents result in more pronounced clinical signs than an infection with the same species of coccidia or nematodes alone (Davis, Herlich, Bowman, 1959, 1960; Catchpole and Harris, 1989). The severity of the disease syndrome may have resulted from enhanced penetration of the mucosa and development of the coccidia following damage caused by nematodes since they occupy the same region.

There are relatively few reports of helminth-induced enhancement of protozoal infections in laboratory studies (Yusuf *et al.*, 1880; Bell *et al.*, 1984a,b; Nichol and Sewell, 1984, Christensen, *et al.*, 1987). An enhanced *B. microti* parasitaemia in concurrent infection with either *Taenia crassiceps* or *T. taeniaeformis* has been observed in CF1 mice (Nichol and Sewell, 1984). *T. spiralis* infection in mice can induce a synergistic influence on *Trypanosoma musculi* to result in elevated and prolonged parasitaemia (Bell *et al.*, 1984a,b; Chiejina, unpublished data). These effects are attributed to immunosuppression by the helminths.

Not all interactions produce the predicted result. For instance, conjoint infection of *T. musculi* with *H. polygyrus*, did not promote the parasitaemia of *T. musculi* over the level of single infection in mice, in spite of the well known immunosuppressive effect of *H. polygyrus* (Bell *et al.*, 1984a). Thus the outcome of concurrent protozoan and helminth infection is still equivocal as it may differ markedly depending on several factors that have still to be elucidated.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 MICE

All experiments were conducted with female Tuck Outbred ('TO') mice purchased from A. Tuck and Sons where they had been raised as specific pathogen (helminth) free animals. They were aged six to eight weeks and weighed 20-25g at the start of each experiment. This strain of mice was chosen to mimic the degree of genetic variability found in natural host populations.

3.1.1 Management of Mice

A maximum of six mice were maintained in a cage with a polypropylene body and stainless steel top, measuring 33x15x13 cm. This always contained a mixture of wood shavings and Okaite spill-dry (BS&S Ltd, Edinburgh) as desiccant. However, during faecal collection, the mice were kept on raised metal mesh which separated them from the bottom of the cage where moist paper towels were placed to collect the faecal pellets. Water and a standard murine feed (SDS Ltd) were provided *ad libitum*. The animals were held in the Departmental animal house where the room temperature was regulated between 21°C (minimum) and 23°C (maximum). The litter was changed every three days and all equipment used was cleaned with jets of hot water.

3.1.2 Infection of Mice with Parasites

The mice were infected orally with *H. polygyrus*. The dose of infective larval was adjusted with distilled water to a volume not exceeding 0.2ml of the suspension. In order to obtain accurate dosage a well mixed larval suspension was administered with an automatic pipette adapted to take a blunted and slightly curved 20-gauge needle.

Inocula of metacyclic or stabilated bloodstream form *T. congolense* were given intraperitoneally in doses adjusted to 0.1ml with phosphate saline glucose (PSG) per mouse, using tuberculin syringe and a 25-gauge hypodermic needle.

3.1.3 Collection and Storage of Sera

Serum samples were obtained after the determination of the packed cell volume (section 3.3.1) or from blood collected from the heart of mice killed at the

end of an experiment. These were stored as individual samples in Eppendorf vials at -20°C.

3.1.4 Chemotherapy

3.1.4.1 Pyrantel embonate

Pyrantel embonate (Strongid - P® granules, Pfizer) was used to terminate adult infections of *H. polygyrus*. Each mouse was treated at the rate of 100mg/kg live weight with anthelmintic in suspension in distilled water, administered orally in a volume not exceeding 0.1ml. This dose rate has been reported to remove all adult *H. polygyrus* from the intestinal lumen of mice (Behnke and Wakelin, 1977).

3.1.4.2 Ivermectin

Ivermectin (Eqvalan® paste, MSD-AGVET) which contained 1.87% w/w anthelmintic was diluted with distilled water to adjust the dose to 20mg/kg body weight in a volume not exceeding 100μl per mouse and given orally. This dose was recommended for treatment of rodent parasites by McKellar (1989). Ivermectin is the 22, 23 dihedral derivative of avermectin B1 which is known to exhibit a broad spectrum of activity against a variety of nematodes at very low doses (Campbell, 1985).

3.1.4.3 Cyclophosphamide

In order to obtain general suppression of the immune response to *T. congolense*, Cyclophosphamide (Sigma) which is an immunodepressant, through its selective cytotoxicity on T-cells, was given at 300mg/kg body weight as a 30mg/ml solution injected intraperitoneally. This treatment was given to mice three hours before infection with *T. congolense*.

3.2 *HELIGMOSOMOIDES POLYGYRUS*

The nematode parasite used, which corresponds to *H. polygyrus bakeri* described by Durette-Desset *et al.* (1972) and Behnke *et al.* (1991), was obtained by courtesy of Dr. F. N. Wahid and Professor D. Wakelin from the Department of Life Science, University of Nottingham, where it has been routinely maintained in CFLP mice.

3.2.1 Faecal Culture

Faecal pellets collected from infected mice were pooled and broken up in distilled water. The suspension was passed through a nylon sieve (mesh size 1 mm) to remove coarse particles, then centrifuged for 5 minutes at 1000g in 50ml test tubes. After syphoning off the supernatant, the sediment was mixed with an appropriate amount of vermiculite to remove excess water before being transferred to Kilner jars. Faecal cultures were left for seven days at room temperature (20-25°C) as this ensured optimal hatching and development of the *H. polygyrus* (Murua, 1975).

3.2.2 Harvest and Preservation of Infective Larvae (L_3)

After six or seven days of culture, the visible columns of L_3 on the sides of the Kilner jar were washed to the base with jets of distilled water from a Pasteur pipette. Relatively clean suspensions of L_3 were obtained from the culture by pipetting from the bottom of the jar. When necessary, further cleaning was carried out by pouring the larval suspension through a single ply of Kleenex® tissue (Kimberly-Clark GmbH) held over a 100ml beaker. Particulate materials were retained in the tissue and the L_3 were recovered from the water in the beaker. Larval suspensions were stored at 4°C in shallow water held in 50ml conical flasks and used used within four weeks of recovery.

3.2.3 Preparation of Doses of Infective Larvae (L_3)

In order to obtain the desired doses of L_3, the numbers of L_3 in each ten 50μl aliquot of the larval suspension were counted under a dissecting microscope. From the average number of L_3 so obtained, the volume of well suspended L_3 was adjusted with distilled water so that the correct numbers of L_3 for administration was contained in less than 0.2ml.

3.2.4 Faecal Egg Counts

One gram of faeces from pooled faeces that had been deposited overnight by all the mice in each group was dispersed in 30ml of saturated sodium chloride solution (SG 1.28). The suspension was passed through a nylon sieve of mesh size

1.0mm and made up to 45ml with additional salt solution. Well mixed aliquots were counted in a standard McMaster counting slide (Hawksley, England) and expressed as eggs per gram (epg) of faeces (MAFF, 1977).

3.2.5 Post mortem Worm and Egg Counts

Each mouse was killed with diethyl ether (May & Baker Ltd, England) and the gastrointestinal tract was removed immediately. The entire length of the small intestine was opened by cutting along its longitudinal axis with a pair of fine scissors. The adult worms were recovered from the intestine by means of a modified Baerman's technique similar to that used by Wakelin and Lloyd (1976) for the recovery of adult *Trichinella spiralis* from mouse intestine. The small intestine was placed in a bag of nylon gauze (mesh size 1mm) which was inserted into a universal bottle containing 25ml of Hanks Balance Salt Solution (HBBS) with antibiotics (100iu penicillin/ml, 100μg streptomycin/ml and 2μg fungizone/ml) at 37°C. Incubation was carried on in a 37°C water bath for 4 hours, by which time virtually all the worms had migrated from the intestine into the fluid outside the bag. The bag and its contents were removed and incubation continued for another 20 hours to ensure complete disentanglement of the worms.

At the end of this incubation, saturated NaCl solution was added to the Hanks solution containing the worms to make up a 30% (v/v) solution. The worms then died within 30 minutes as a result of this treatment, relaxing the tight spiral coils characteristic of the living worms, thus making counting and subsequent measurement easier.

The universal bottle was then filled with saturated NaCl solution to form a positive meniscus. The worms remained at the bottom but the eggs which had been passed *in vitro* during the previous 24 hours floated up and were aspirated off. The aspirate was then made to 45ml with saturated NaCl solution and the eggs counted using the McMaster slide. Counts were expressed as eggs per female per 24 hours.

Worms from each mouse were transferred into a ruled Petri dish, sexed and counted under a binocular dissecting microscope (Wild Heerbrugg).

3.2.6 Measurement of Worms

Female and male specimens of adult worm samples were picked out with fine Swiss tweezers (TAAB, Reading), were mounted in lactophenol (BDH) on a microscope slide and a covered with coverslip. Worms, at magnification of x150 on a dissecting microscope, were projected through a colour television camera (Panasonic F10) to a colour video monitor (Barco, DCD 1640F). Worms were traced from the screen of the monitor onto acetate sheets using a marking pen. The length of each individual worm was determined from tracings with a calibrated map reader having a finely toothed wheel.

3.2.7 Acid-Pepsin Digestion

Intestinal tissues from individual mice were digested in 500ml of a mixture of pepsin/hydrochloric acid, pH 2.0 (i.e. 0.5% w/v pepsin (EC 3.4.23.1), Sigma, and 0.5% hydrochloric acid in distilled water) at 37°C as described by Wahid and Behnke (1992) except that the digestion time was increased to 12 hours. The digest was cleaned by successive washing and sedimentation and fixed with 10% v/v formalin in distilled water to be preserved until any larval stage present could be enumerated under a dissecting microscope.

3.3 *TRYPANOSOMA CONGOLENSE*

The strains of *T. congolense* used were obtained from the stabilate bank of the Centre for Tropical Veterinary Medicine by the courtesy of Dr. Carole A. Ross and colleagues. These stabilates are all given coded numbers with the prefix TREU (Trypanosome Research Edinburgh University). The two stabilates used in this work were TREU 1457, a cloned derivative of TREU 1290 which was originally isolated from an ox in Zaria, Nigeria in 1967 as Zaria/67/LUMP/69 (Luckins and Gray, 1983), and TREU 1881, one of the cloned derivatives of TREU 1842 which was initially isolated from dogs in Zambia in 1981 (DA/ZM/81/TRPZ

105) and then inoculated into Wistar rats before subpassaging in mice (Frame *et al.*, 1990).

3.3.1 The *in vitro* Culture System

In vitro cultures of the insect forms of *T. congolense* (TREU 1457 and TREU 1881) were initiated in 25cm^2 (50.0ml) flasks (Nunclon, Gibco Europe Ltd, Paisley) according to the method of Gray *et al.* (1984) except that the flasks were gassed with 5% CO_2 in air before they were seeded with 1.0ml of supernatant of a parent culture. A fresh growth medium prepared from a powdered form of Eagle's Minimum Essential Medium (Gibco Europe Ltd) supplemented with 4mM glutamine, 20mM HEPES buffer, 2.2g/l sodium bicarbonate and 20% (v/v) foetal calf serum which was heat inactivated at 56°C for 30 minutes before use, was then added to make 4.0ml. Cultures were maintained at 28°C in 4.0ml of medium which was changed at 48h intervals. After 14 days, the epimastigotes differentiated into metacyclic trypanosomes, yielding 1 - 1.7 x 10^7 organisms /ml of culture supernatant, as determined by counts in a haemocytometer (Hawksley, England).

3.3.2 Separation of Trypanosomes

Cultured metacyclic trypanosomes were separated from the epimastigotes by passing approximately 4.0ml of freshly harvested culture supernatant through an equivalent volume of packed column of diethylamoethyl-cellulose (Whatman DE 52, Whatman Labsales, England) equilibrated with phosphate buffered saline pH 8.0 containing 1% w/v glucose (PSG), as described by Gray *et al.* (1984). Bloodstream forms from infected animals were also separated from heparinized blood by this same chromatographic technique, but five times the equivalent amount of DE 52 was used. These trypanosomes were eluted with PSG, collected in sterile universal bottles and then centrifuged at 2260g for 20 minutes at 4°C. The packed organisms were resuspended in PSG and adjusted to the required concentration after the total eluate has been obtained by counting on a haemocytometer (Hawksley, England).

3.3.3 Cryopreservation and Resuscitation of Metacyclic Forms

As it was necessary to prepare and hold large quantities of the stabilates to be used during the project, two mature cultures were scraped off the bottom of the flasks into the supernatant medium with a sterile Cell Scraper (Costar Coporation, USA). The entire suspension was pipetted into a sterile universal bottle and an equal volume of fresh medium, previously made up to 15% (v/v) with sterile prewarmed glycerol (37°C) was added. The trypanosome suspension was then dispensed in aliquots of 1.0ml into sterile vials and cryopreserved using a programmable Cell Freezer (Planer Products Ltd). The freezing cycle used (programme 9) was as follows:

stage 1	-5°C/min. to +4°C
stage 2	HOLD 5 minutes
stage 3	-1°C/min. to -30°C
stage 4	-2°C/min. to -60°C
	HOLD.

After freezing the trypanosomes were transferred into liquid nitrogen for storage in the liquid phase.

Stabilated cultures were resuscitated by quick thawing in water bath at 30°C before being used to initiate *in vitro* cultures in 25cm^2 flasks as described in section 3.3.1.

3.3.4 Cryopreservation of Bloodstream Forms

Mice carrying infection arising from metacyclic infection were bled to make cryopreserved stabilates. An equal volume of the cryoprotectant solution (15% of Dimethyl Sulphoxide (DMSO) (Sigma) in Phosphate Saline Glucose (PSG)) was added dropwise and mixed gently with the heparinised blood which had been on ice (the final concentration of DMSO being 7.5%). Cryopreservation was done by transferring the stabilates in 2ml polypropylene vials (in insulating boxes) to solid carbon dioxide (dry ice) for 24 hours, then to liquid nitrogen. After 24 hours one capillary was brought out and checked for viability.

3.4 HAEMATOLOGY

3.4.1 *Trypanosoma congolense* Parasitaemia

Mice were bled from the tail at intervals for the estimation of parasitaemia according to the method of Herbert and Lumsden (1976). At higher levels of infection, microscopic fields (x400 magnification) of wet blood film were matched against standard charts and, where fewer organisms were present, the number of trypanosomes in 5, 10, or 20 such microscope fields were counted and the equivalent number in one millilitre of blood was read from a standard table (Herbert and Lumsden, 1976). The estimated number of organisms per field were expressed as base $\log_{10}$ per millilitre of blood.

3.4.2 Packed Red Cell Volume

Blood was collected from the tails of mice into disposable heparinized capillary tubes (Camlab Ltd, Cambridge). The tubes were filled up to two-third full and sealed at one end with Cristaseal (Hawksley, England). The percentage of packed red cell volume (PCV) of individual mice was determined by a haematocrit method. The tubes were placed in a microhaematocrit centrifuge (Haemofuge A, Heraeus Sepatech) and centrifuged for five minutes at 17,000g. The PCV was determined using a microhaematocrit reader (Heraeus Reader)

3.4.3 Differential Leucocyte Counts

Thin blood smears were made on clean microscope slides and then air dried and subsequently stained with Leishman stain (Gurr®, BDH Ltd, Poole England) for 2 minutes. The stain was then diluted to 50% (v/v) with Leishman's stain buffer solution (pH 6.8) and allowed to stand for 8 minutes before being washed off with buffer. The smears were then blotted dried. Alternatively, in some experiments, dried blood smears were fixed in methanol for two minutes and then stained for 30 minutes in 10% (v/v) Giemsa stain (BDH) diluted with distilled water.. The slides were washed with Giemsa buffer (BDH) pH 7.2 and allowed to drip dry.

Differential leucocyte counts were performed on the stained slides by counting a total of 100 cells in successive microscope fields using the oil x100 objective on a Leitz Dialux 22 microscope (Leica U.K. Ltd).

3.5 HISTOLOGY

Those portions of the gastrointestinal tract intended for histological studies were fixed in 10% (v/v) formalin immediately after removal from the host. The fixed samples were trimmed, embedded in paraffin wax block and cut into thin 4μ longitudinal and transverse sections with a microtome by standard methods. Paraffin sections were stained with haematoxylin and eosin (H&E) stain or by the Periodic Acid-Schiff (PAS) reaction to demonstrate mucin (Bancroft and Stevens, 1977).

3.6 PARASITE EXTRACTS

Various parasite extracts of *H. polygyrus* and *T. congolense* were prepared and used as antigens in Antibody Enzyme Linked Immunosorbent Assay (ELISA).

3.6.1 Adult Surface Extract of *Heligmosomoides polygyrus*

Mature adult worms were extracted postmortem from the intestines of infected mice 14 days after infection as described in section 3.2.5. Worms were then washed five times in 10.0ml of sterile saline containing 100iu penicillin/ml, 100μg streptomycin/ml and 2μg fungizone/ml. In order to obtain detergent surface extract worms were gently mixed in 2ml of either 1% (v/v) solution of n-octyl B-d-glucopyranoside (NOG) or 0.25% (w/v) of Cetyltrimethyl-ammonium bromide (CTAB) in a mixture of protease inhibitors in PBS (PBS-PI) for 30 minutes on an ice bath. The PBS-PI contained N-α--Tosyl-L-lysine chloromethyl ketone (50μg/ml), L-1-Tosyl-amide-2-phenyl-ethylchloro-methyl ketone (25μg/ml), phenylmethyl-sulphanyl fluoride (1mM) and 1% (w/v) sodium deoxycholate.

The supernatant extract was transferred into 15mm (diameter) dialysis tubing (Sigma) previously shown to be leak free and dialysed overnight at 4°C. The NOG extract was dialysed against 70ml of PBS-PI so that the final

concentration of NOG was 0.03% v/v dialysed state and CTAB extract was dialysed against 16.7ml of PBS-PI. The protein content of the surface extracts were determined as described in section 3.7 and stored in 20-μl aliquots at -70°C.

3.6.2 Somatic Extract from Adult *Heligmosomoides polygyrus*

A somatic extract was obtained from mature adult worms recovered *post mortem* from infected mice. 0.5ml of packed adult worms obtained from mice and washed in antibiotic solution as described in section 3.6.1, were pulverised in a sterile glass homogeniser on ice with 2.0ml of PBS-PI (i.e. 1 volume of parasite to 4 volumes buffer). The homogenate was placed on ice for a further 10 minutes and shaken at intervals. It was then put into sterile Eppendorf tubes and centrifuged for 20 minutes at 25,848g at 4°C . Pooled supernatants from this centrifugation were kept on ice while the protein content was determined as described in Section 3.7 and stored in 20-μl aliquots at -70°C.

3.6.3 Extracts from Metacyclic *Trypanosoma congolense*

Metacyclic *T. congolense* were obtained from *in vitro* cultures established in seven flasks as described in Section 3.3.1. During the routine change of culture medium metacyclic trypanosomes from all the flasks were separated as previously described (Section 3.3.2), pooled and stored in PBS-PI at -20°C until the collection reached 250μl of packed parasites. This was suspended 1.0ml of PBS-PI a plastic Eppendorf tube and sonicated in an ultrasonicator (MSE) at 6-8 microns amplitude for 15 seconds x4 on ice. The sonicated extract was then centrifuged for 20 minutes at 25,848g at 4°C and the protein content determined as described in Section 3.7 and stored in aliquots of 20μl at -80°C.

3.6.4 Extracts from bloodstream forms of *Trypanosoma congolense*

Ten cyclophosphamide-treated mice were infected with 10^4 organisms from a stabilate of TREU 1881. The mice were killed and bled out 10 days later when they had fulminating parasitaemias. The parasites were separated from cellular elements of blood by an anion-exchange chromatography using DE 52 as described in Section 3.3.2. The separated trypanosomes were pelleted by

centrifugation (17,000g) at 4°C and later diluted at 1:4 with PBS-PI and processed as described in Section 3.6.3.

3.6.5 Protein Estimation

The protein content of parasite extracts was estimated according to the method of Warburgh and Christian (1941). Two ml of the extract, diluted to 1:50 with PBS-PI, were prepared and its extinction was measured at both both 260nm and 280nm on a spectrometer (Unicam 8625 UV/VIS). The ratio of E280/E260 was calculated and with this ratio the factor for calculating the protein content was read off from the standard table (Warburg and Christian, 1941). The protein concentration (mg/ml) of the diluted extract was obtained from the following formula:

$$E_{280} \times \text{Factor} \times 1/d$$

d = length of the light path

3.7 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

An ELISA technique was developed and standardised essentially as described by Voller, Bidwell and Bartlett (1979) to monitor antibody responses of mice. A 96-well flat bottom, protein binding rigid polystyrene, microtitration plate (Immulon1, Dynatech Laboratories Inc. Virginia) was coated with 50μl/well of antigens diluted in Borate buffered saline (BBS), pH 8.2. The plate was wrapped with cling film to prevent evaporation and incubated overnight at 4°C. It was then washed twice with saline/Tween (0.15M NaCl/0.05% v/v polyoxyethylene sorbitan monolaurate (Tween 20), Sigma), allowing each wash to stand for 3 minutes. The plate was dried by shaking onto a pack of paper towels.

100μl of blocking buffer made of 4% v/v Normal Goat Serum (NGS) (Gibco) in PBS containing 0.5% w/v of Tween 20 (NGS/PBS/Tween) were added to each well and allowed to stand for one hour at room temperature to block any nonspecifically reactive sites. After shaking out the blocking solution, 50 μl of each serum sample diluted in NGS/PBS/Tween 20 to the required dilution was added to each well and the plates were covered to avoid evaporation and incubated

for 1 hour at 37°C. Each plate included samples of suitably diluted sera from known infected and non-infected mice as controls.

After this incubation and further three 3-minute washes as described above, 50μl of enzyme-conjugate (Goat anti-mouse (heavy and light chain) IgG/Peroxidase [GAM/PO] - IgG whole molecule, Nordic Immunological Laboratories, The Netherlands) diluted to the optimum concentration of 1:2000 in NGS/PBS/Tween, was added to each well. The plate was then incubated for another hour at 37°C and again washed three times as above.

The colour reaction was then developed for 15 minutes at 37°C by the addition of 50μl of the substrate, 3,3',5,5'-Tetramethylbenzidine (Sigma) diluted in 0.05M Phosphate-citrate buffer, pH 5.0, containing 0.014% v/v urea H_2O_2 (Sigma), to each well. The reaction was stopped by the addition of 50μl of 0.02M H_2SO_4 (Aristar, BDH) to each well and the optical density at 450nm was read on a Titertek Multiscan (Labsystems) at 450nm.

ELISA values for the control positive (for either *H. polygyrus* or *T. congolense*) and a negative sera which were included on each test plate were used to standardize ELISA values from plate to plate as follows:

$$\text{Corrected absorbance} = [T_n - N_n] \times [(P_1 - N_1)/(P_n - N_n)]$$

where T_n	test serum assayed on plate n
N_n	negative serum assayed on plate n
P_n	positive serum assayed on plate n
N_1	negative serum assayed on plate 1
P_1	positive serum assayed on plate 1

3.7.1 ELISA Titrations

ELISAs were carried out to determine the optimum dilutions for parasite antigens, enzyme conjugate and serum samples. A positive serum against *H. polygyrus*, which consisted of pooled serum samples from mice infected with 500 L_3, treated six days later with ivermectin and bled 30 days after treatment, were used for this titration and as a positive control in subsequent studies. Similar titrations were carried with the pooled sera from mice with a 70-day old *T.*

congolense infection which was used throughout the study to provide the known positive control for determining antibody response against the trypanosome antigens. Serum pooled from 10 uninfected mice was used as the negative control throughout the study.

3.8 STATISTICS

The results are presented as mean values $\pm$ standard error (SEM). Non-parametric statistical analysis were used to analyse data sets with small sample sizes (Fowler and Cohen, 1990). When more than two groups required comparison at a single time point, the Kruskal Wallis statistic H was calculated to determine whether there was a significant treatment effect. If significant, two groups as stated were compared by the Mann-Whitney U test for significant differences. Where appropriate, data were compared by a one-way analysis of variance (ANOVA). The relationship (rectilinear) between two variables was tested by least squares linear regression analysis and the correlation coefficient, r, given as appropriate. Differences were considered significantly different at $P<0.05$. Statistical procedures were carried out with Minitab statistical software (Minitab, Inc. USA) and with Instat (GraphPAD Software Inc., USA).

CHAPTER FOUR

INFECTION OF FEMALE OUTBRED MICE WITH METACYCLIC AND BLOODSTREAM FORMS OF *TRYPANOSOMA CONGOLENSE*

4.1 INTRODUCTION

Metacyclic trypanosome forms are the infective population that initiates natural infections. Patent infections in some laboratory animals have been initiated with metacyclic forms after either trypanosome-infected tsetse fly bite (Luckins, Hopkins, Rae and Ross, 1990) or needle challenge (Mwangi, 1991). *In vitro* culture systems now provide reliable sources of large numbers of metacyclic trypanosomes which retain both the morphological and biological characteristics of trypanosomes found in the tsetse fly. Studies by Luckins, Frame, Gray, Crowe and Ross (1986) indicated that there are no changes in expression of metacyclic variable antigenic types (M-VATs) in cultures of *T. congolense* even after several passages, cryopreservation or long-term cultivation *in vitro*. Moreover, metacyclic trypanosome populations produced over long periods in culture remain relatively stable antigenically (Gray and Luckins, 1982). Such stable metacyclic antigen profiles, characteristic of cultured metacyclic trypanosomes could be of value in initiating uniform infections in animals during controlled laboratory studies. There are no reported studies on the use of cultured metacyclic trypanosomes in the study of the dynamics of trypanosomosis in the laboratory. The use of culture derived metacyclic trypanosomes was explored in the hope of recreating the natural situation in the laboratory through chronic infections characteristic of most field infections. The following experiments were carried out to obtain baseline information for *T. congolense* infection with regards to (1) suitable levels of infection to be used in subsequent studies, and (2) the responses of female out-bred 'TO' mice to infections initiated with either the metacyclic *T. congolense* obtained from *in vitro* cultures or stabilated bloodstream forms of this trypanosome.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Animals

Female out-bred 'TO' mice aged six to eight weeks were obtained from A. Tuck and Sons and managed as described under Section 3.1.1.

4.2.2 Parasites

The first set of experiments were carried out with metacyclic trypanosomes of TREU 1457 and TREU 1881 produced *in vitro*. Later experiments were conducted with stabilated bloodstream forms of TREU 1881. The history of these cloned stocks of *T. congolense* available at the Centre for Tropical Veterinary Medicine has been given in Section 3.3. Each mouse was infected intraperitoneally with 0.1ml of the trypanosome suspension prepared as described in Section 3.1.2.

4.2.3 Experimental Design and Procedures

Several pilot experiments were carried out. These involved infection of the mice with different estimated doses of parasite preparations and monitoring the parasitaemias as described under Section 3.4.1.

4.2.3.1 Experiment 4.1

18 females were randomly sorted into three groups (TC1, TC2 and TC3) of 6 mice each and infected intraperitoneally with 10^3, 10^5 and $2x10^5$ metacyclics of TREU 1457 respectively. The parasitaemia and survival were monitored daily for not less than 30 days after infection.

4.2.3.2 Experiment 4.2

Metacyclic trypanosomes were obtained from cultures of a different stock, TREU 1881, and 18 mice similar to those in Experiment 4.1 placed into three groups (TC6, TC7 and TC8) which were infected with 10^3, 10^4 and 10^5 metacyclic trypanosomes respectively. The same parameters were determined as for Experiment 1 for not less than 30 days after infection.

4.2.3.3 Experiment 4.3

To investigate the reproducibility of the results obtained from Experiment 4.2, groups TC9, TC10 and TC11 were infected with 10^3, $2x10^3$ and 10^4 metacyclics of TREU 1881 respectively. The same parameters were determined as in the earlier experiments not less than 30 days after infection.

4.2.3.4 Experiment 4.4

Further replications of infections with 10^5 and 10^3 metacyclics were tried in similar groups of mice, TC12 and TC13 respectively. The same parameters as in the earlier experiments were again determined till 30 days after infection.

4.2.3.5 Experiment 4.5

Fresh cryopreserved stabilates of TREU 1881 were resuscitated to initiate new cultures from which further titrated doses of infections were investigated. Groups of mice designated TC16, TC17, TC18 and UC4 were infected with 10^3, 10^4, 10^5 and nil metacyclic trypanosomes respectively. The same parameters as in Section 4.2.3.1 were monitored for no less than 30 days post infection.

4.2.3.6 Experiment 4.6

The use of another preparation of *T. congolense* consisting of cryopreserved bloodstream forms of TREU 1881 obtained from the first parasitaemic peak in mice previously immunosuppressed with 300mg/kg body weight cyclophosphamide were explored.

Freshly thawed vials of parasites were quickly diluted and administered in 0.1ml suspensions (Section 3.1.2). Following an initial pilot study involving 2 animals in each group (TC19), 5 groups of 6 mice each, TC20, TC21, TC22, TC23 and UC6 were infected with 10^2, 10^3, 10^4, 10^5 and nil bloodstream forms respectively. In addition to assessing the parameters as in Section 4.2.3.1, the PCV (Section 3.4.2) and the live weights of each mouse were monitored weekly. Serum samples were collected weekly from each mouse as described in Section 3.1.3. for the measurement of antibody response by ELISA (see Section 3.7).

4.3 RESULTS

4.3.1 Response to Metacyclic Trypanosomes

Initial experiments were carried out with TREU 1457 (Section 4.2.3.1) but it was not possible to carry out a repeat titration infection due to the collapse of the culture system involving this stock. The results obtained with regards to

parasitaemia and mortality due to this stock are presented (Figure 1a,b). The results thus presented here are those involving TREU 1881, cultures of which consistently produced large quantities of metacyclic trypanosomes for the series of titration experiments.

4.3.1.1 Parasitaemia

The time to patency from infection varied from 2 to 14 days. Some inocula (Experiments 4.3-4.5; Figures 4.3a, 4.4a, 4.5a) resulted in nil or only a fleeting parasitaemia. The dose response in terms of parasitaemia was inconsistent although the peak parasitaemias were generally observed approximately two weeks after infection Figures 4.1a- 4.5a).

4.3.1.2 Mortality

Mortality was one consequence of infection with *T. congolense* (Figures 4.2b - 4.5b). Peak parasitaemias were generally followed by a sharp drop in the number of surviving mice. This was particularly exemplified in Experiment 4.2 where all mice died before 21 days after infection (DAI).

4.3.2 Response to Bloodstream Form Trypanosomes

4.3.2.1 Parasitaemia

Patency was reached within four to 6 DAI (Figure 4.6a) and the peak parasitaemia was obtained between 8 and 10 DAI. However, only 50% of the mice infected with 10^3 organisms were infected. The first parasitaemic peak in mice infected with 10^4 - 10^5 organisms occurred at 9 DAI while parasitaemia in those infected with 10^3 and 10^4 peaked at days 13 and 11 respectively.

4.3.2.2 Mortality

At least 50% of the infected mice survived until 30 DAI when the experiment was terminated (Figure 4.6b). The first deaths were recorded at 17, 11 and 10 DAI in the groups infected with 10^3, 10^4 and 10^5 organisms respectively. All control mice and those infected with 10^2 organisms survived till the end of the investigation.

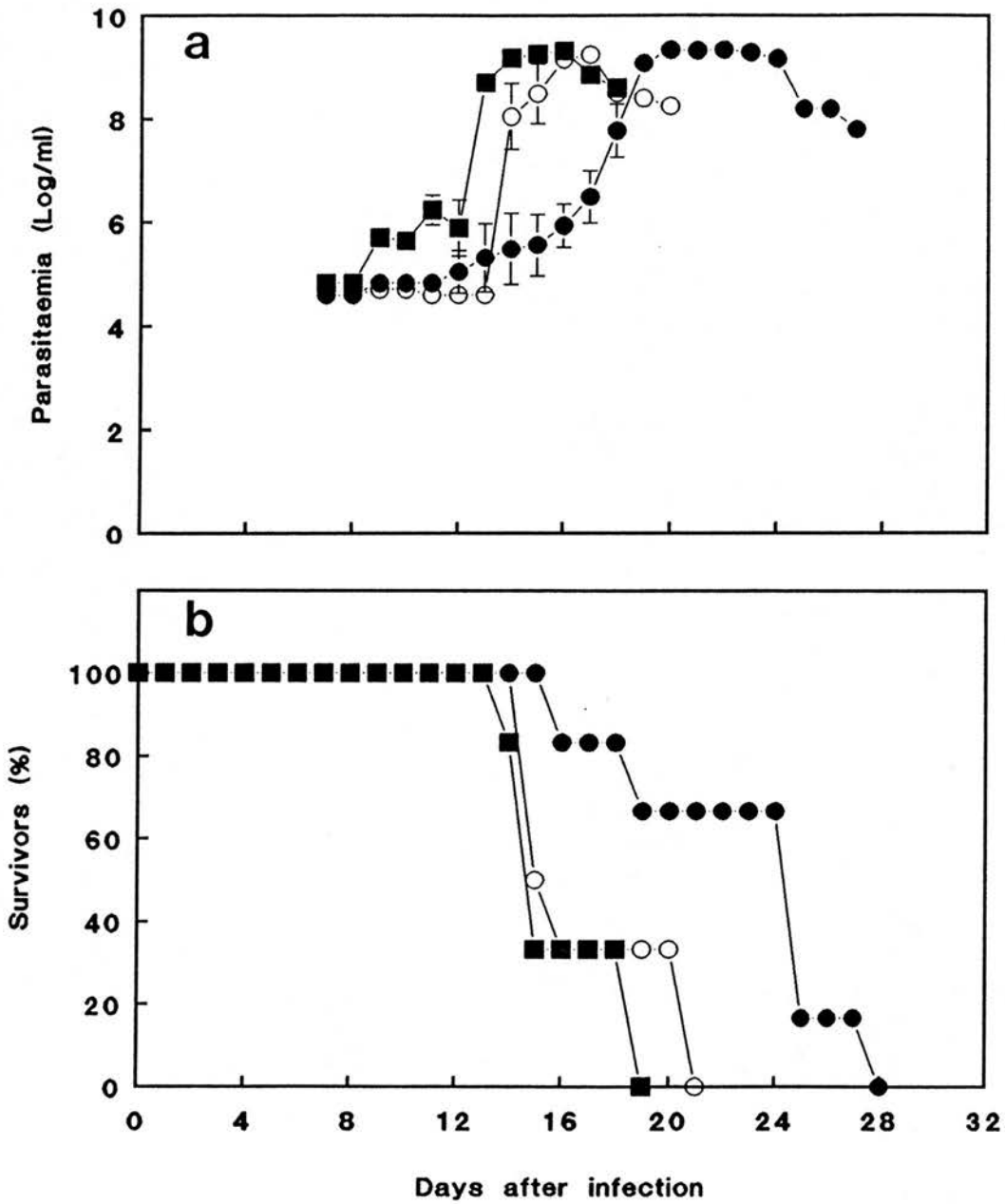

FIGURE 4.1 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^3 (●), 10^5 (○) and $2x10^5$ (■) metacyclic trypanosomes of TREU 1457 (Experiment 4.1).

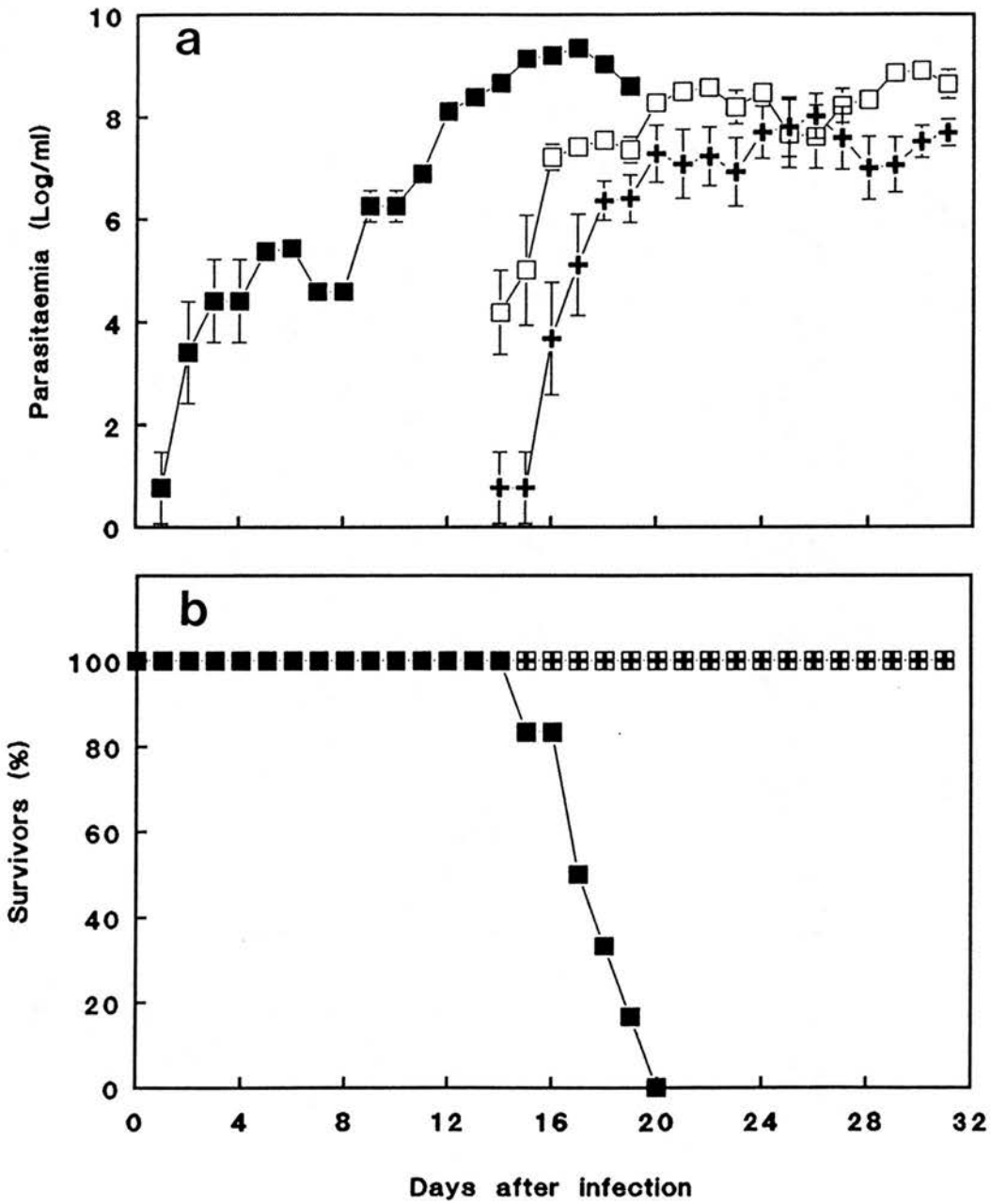

FIGURE 4.2 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^3 (⊞), 10^4 (□) and 10^5 (■) metacyclic trypanosomes of TREU 1881 (Experiment 4.2).

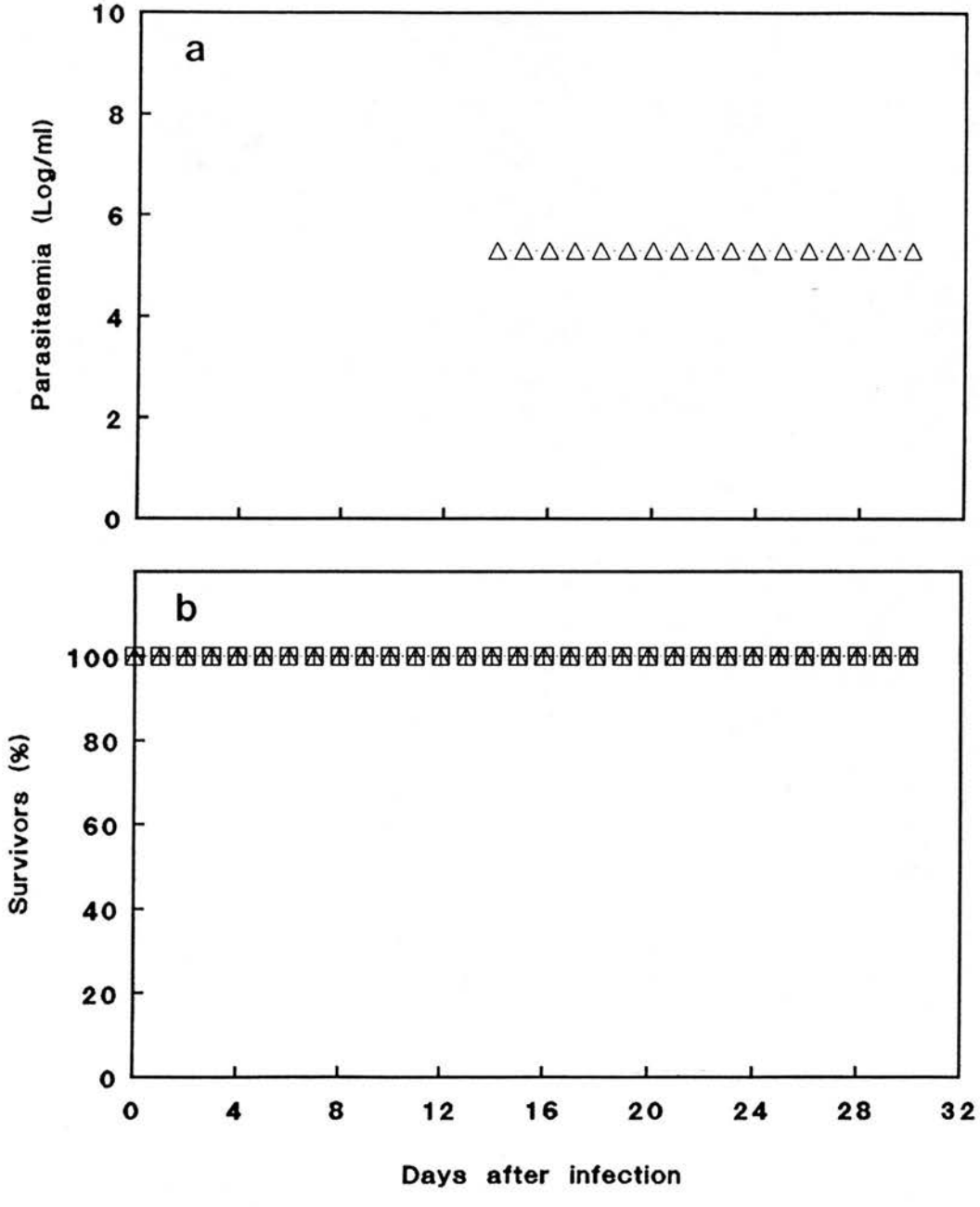

FIGURE 4.3 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^3 (△), $2x10^3$ (□) and 10^4 (+) metacyclic trypanosomes of TREU 1881 (Experiment 4.3).

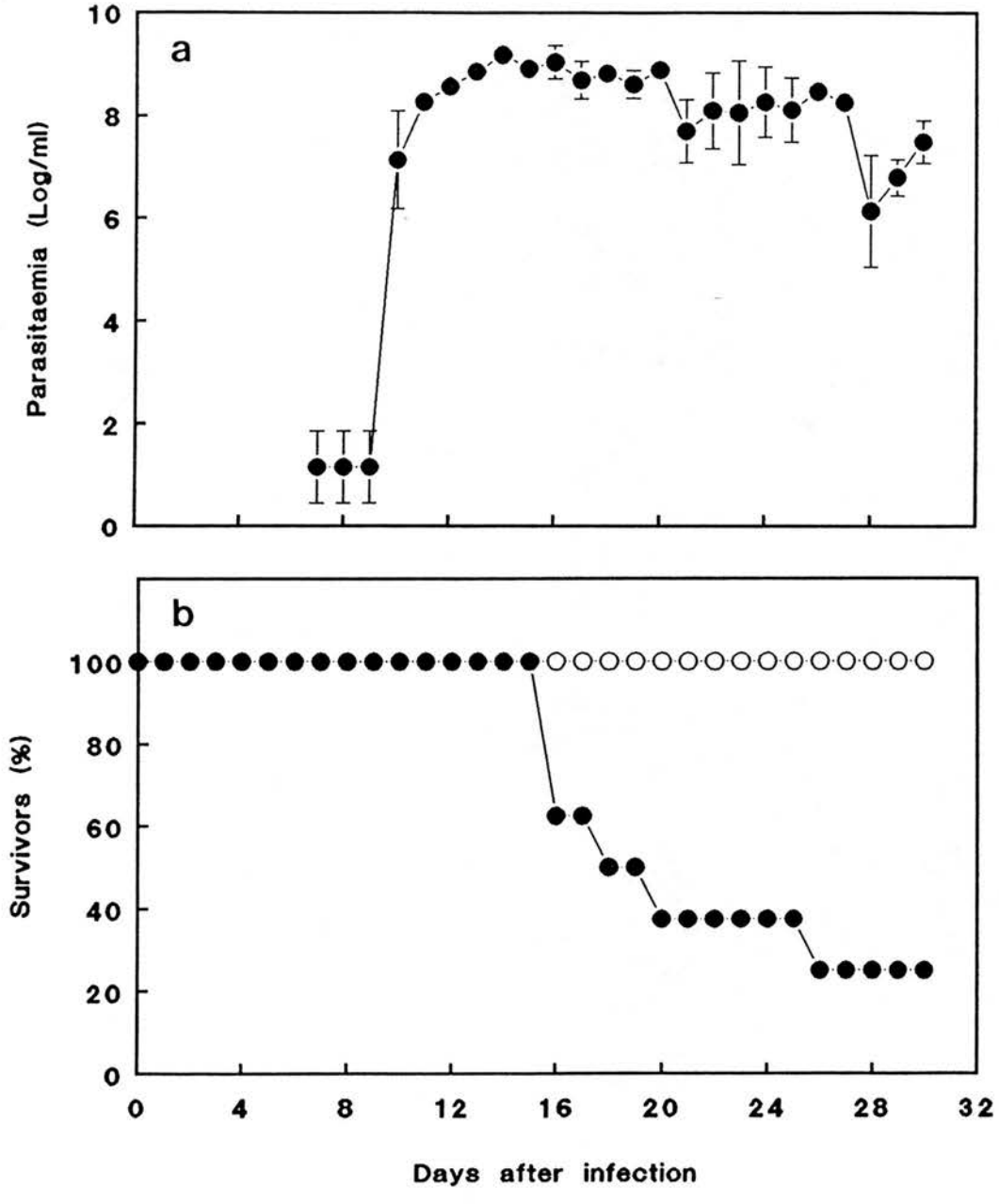

FIGURE 4.4 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^5 (●)and 10^3 (○) metacyclic trypanosomes of TREU 1881 (Experiment 4.4).

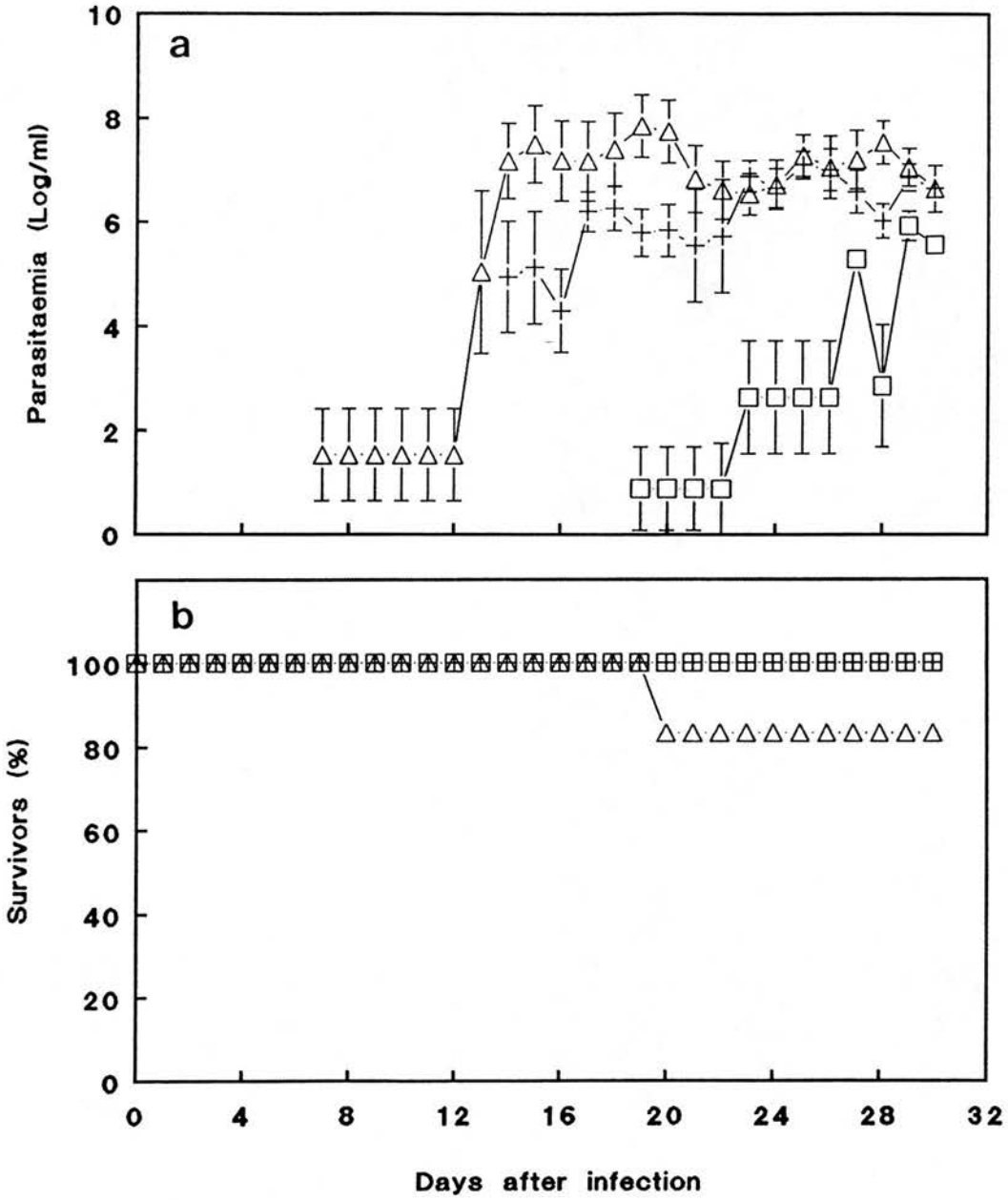

FIGURE 4.5 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^3 (□), 10^4 (+) and 10^5 (△) metacyclic trypanosomes of TREU 1881 (Experiment 4.5).

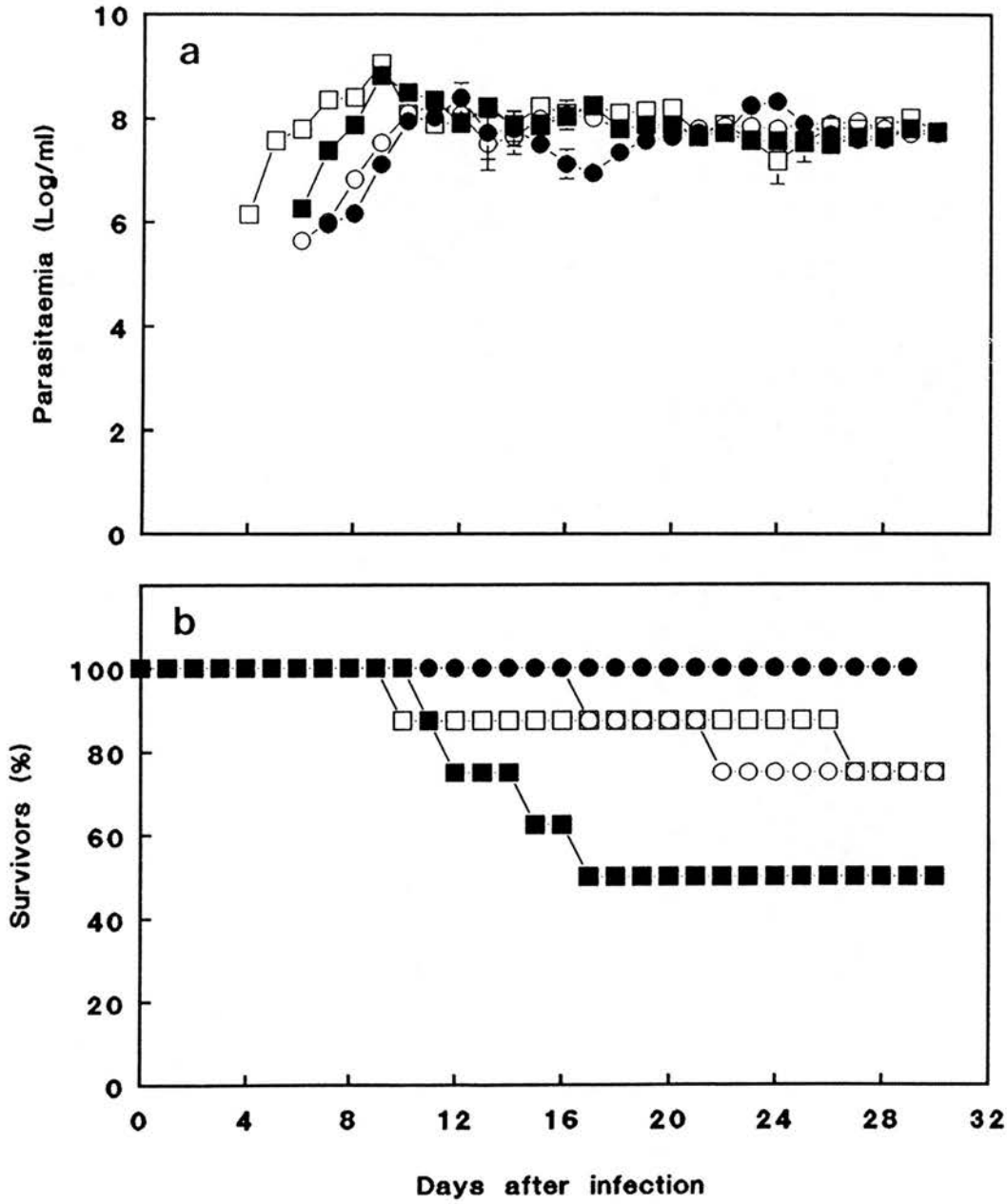

FIGURE 4.6 The mean (±SEM) parasitaemia (a) and the survival rate (b) in mice infected with 10^2 (●), 10^3 (○), 10^4 (■) and 10^6 (□) bloodstream forms of TREU 1881 (Experiment 4.6).

4.3.2.3 Packed cell volume

Infection with trypanosomes was generally accompanied by a drop in the PCV of the mice (Figure 4.7a). From 14 DAI, the mice infected with 10^2 organisms gradually recovered and, at 28 DAI, they had similar PCV values with the uninfected control group (U=21.5, P>0.05), but those infected with 10^3, 10^4 and 10^5 organisms had significantly lower PCVs, than the control (U=0, 0 and 1 respectively, P<0.05).

4.3.1.4 Live weight of mice

Figure 4.7b shows the weekly live weights of the infected and uninfected (control) mice. All groups of infected and uninfected mice generally gained weight and had similar weights up to 14 DAI. Although the weight of the mice in the group infected with 10^4 trypanosomes was slightly higher than the other groups from 21 to 28 DAI, there was no significant difference between the groups (H=5.35, P=0.253).

4.3.1.5 Antibody response to infection

Following ELISA test titrations (Section 3.7.1) with known positive anti-*T. congolense* and negative serum samples, the ELISA values obtained were plotted to determine the optimum dilutions for parasite antigens, enzyme conjugate and serum samples. Based on the optimum values obtained from the titration curves (Figures 4.8a,b,c) the test ELISA plates were coated with antigen at a protein concentration of 5μg/ml, the conjugate was used at a dilution of 1:2000, and the test sera were used at 1:50.

The ELISA technique described under Section 3.7 was used to detect antibodies specific for *T. congolense* in samples of mouse plasma. Samples were examined in duplicate. ELISA values for each animal on each occasion consisted of the average of results obtained using its serum, while the values for each group represent the means for the individual mice corrected as described in Section 3.7.

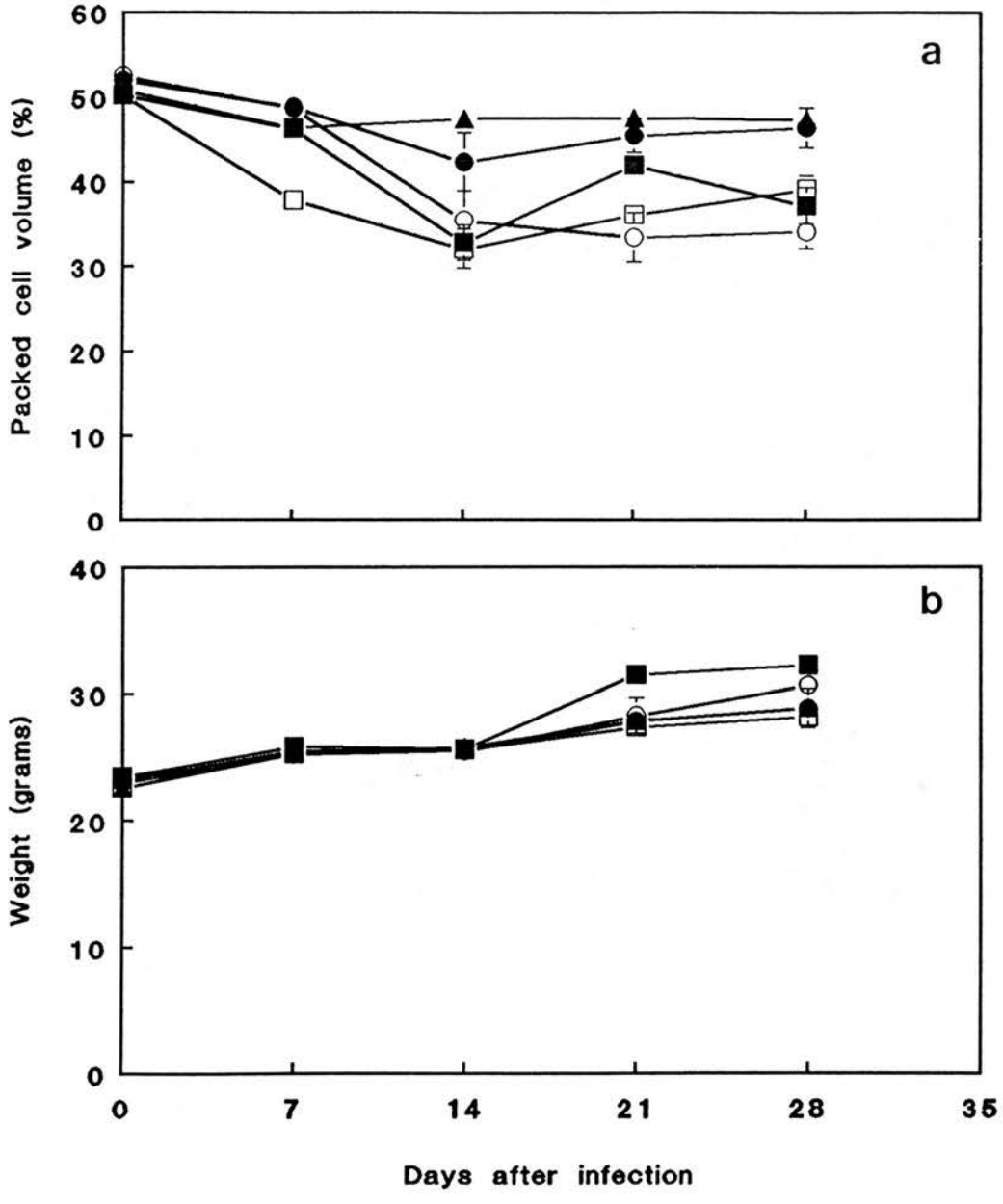

FIGURE 4.7 The mean (±SEM) weekly PCV (a) and live weight (b) uninfected mice (▲) and those infected with 10^2 (●), 10^3 (○), 10^4 (■) and 10^6 (□) bloodstream forms of *T. congolense* (TREU 1881) (Experiment 4.6).

The antibody response in the mice in Experiment 4.6 is presented in Figure 4.9. The response to infection occurred earlier (7 DAI) in the groups infected with 10^4 and 10^5 organisms. However by 14 DAI all the infected mice showed similar responses which increased with time until the end of the experiment.

4.4 DISCUSSION

The results of experiments with metacyclic trypanosomes in TO mice demonstrated that the dynamics of infections obtained from inoculations with this form of the parasite were inconsistent and this necessitated the repeated titration experiments. Doubts that the inconsistency was due to ageing cultures were dispelled by the use of fresh cultures in Experiment 5. Culture derived metacyclic forms of *T. congolense* have been used in experimental infections in cattle (Akol, Murray, Hirumi, Hirumi and Moloo, 1986), rabbit (Luckins, Rae and Gray, 1981) and sheep (Mwangi, 1991) but in these cases, the animals were infected by the intradermal route where the trypanosomes undergo a phase of extravascular multiplication in the collagen during the first seven to 9 days of the infection. Thus the skin not only acts as a focus for the establishment of the infection but also as a site for localised proliferation of the parasite before dissemination into the blood system (Akol and Murray, 1982). The reason for the unpredictable pattern of infection with the metacyclic form of *T. congolense* is not clear since it has been shown that culturing does not alter the antigenicity of the parasite (Gray and Luckins, 1982). It is possible that the manipulations such as extraction from in vitro culture and dilution, affected the viability of the parasite thus contributing to the irreproducibility of the results and the difficulty of obtaining the desired chronic infection. Unlike the bloodstream forms, it was not possible to use representative organisms from one large preparation (such as a stabilate) each time a new infection was initiated. Also, since the intradermal phase is an important element in infection with metacyclic trypanosomes, the route of administration

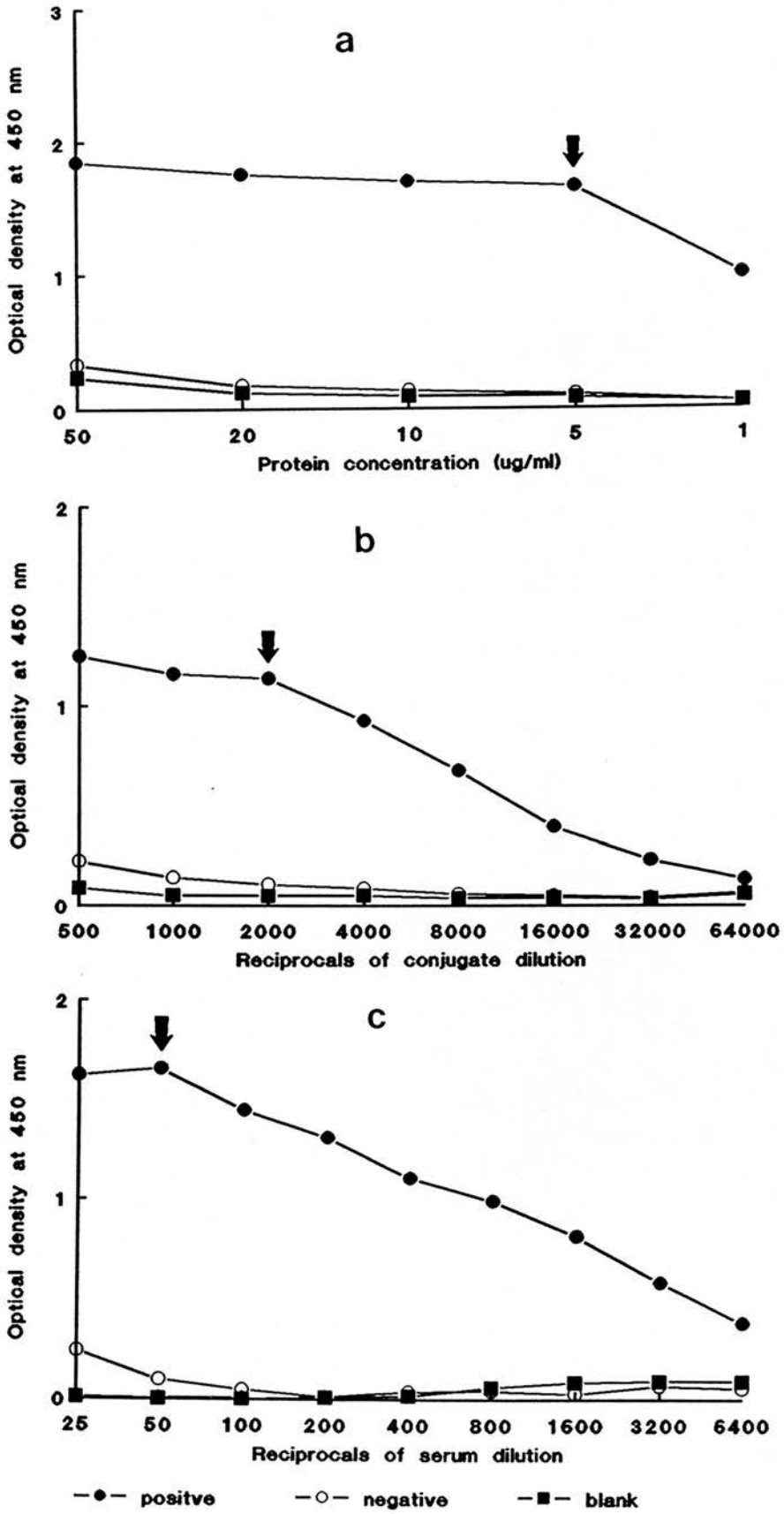

FIGURE 4.8 Results of ELISA titrations carried out with known positive against *T. congolense* and negative samples to determine the optimum (a) parasite antigen protein concentration, (b) enzyme conjugate (goat anti-mouse peroxidase) dilution and (c) · · dilutions for serum samples. The arrows indicate the optima deduced from the curves.

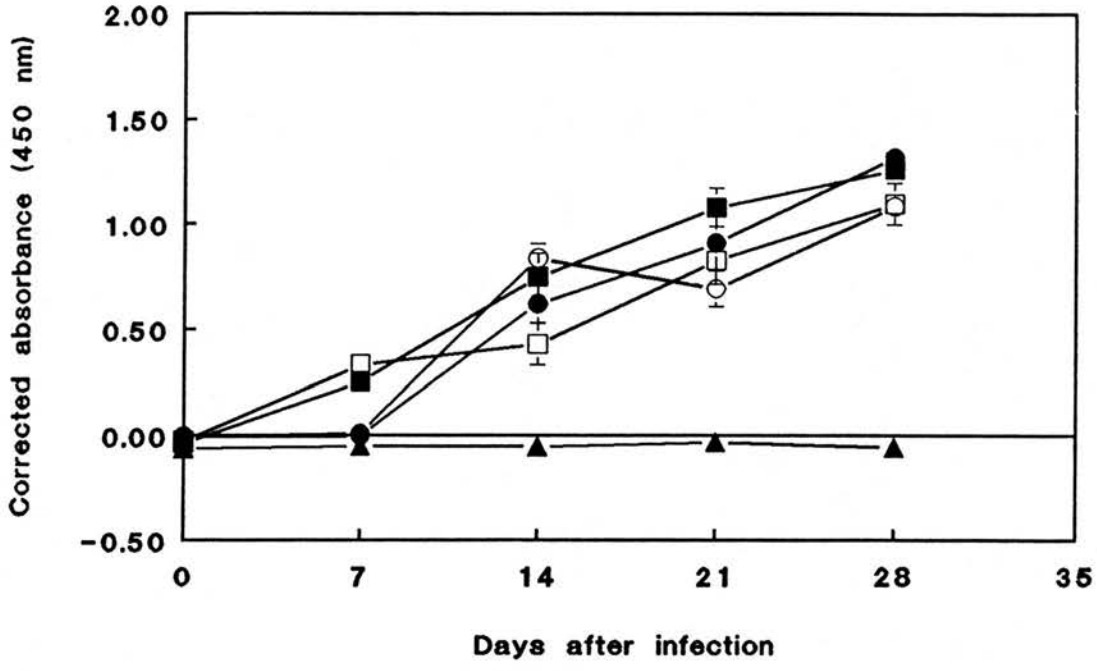

FIGURE 4.9 Mean (±SEM) antibody response of uninfected mice (▲) and those infected with 10^2 (●), 10^3 (○), 10^4 (■) and $+10^6$ (□) bloodstream forms of TREU 1881, to *T. congolense* antigens (Experiment 4.6).

used in the present study may have adversely influenced the outcome of infections. The bloodstream form of *T. congolense* does not require any intradermal development and will have passed from the peritoneum directly into the bloodstream in lymph via the thoracic duct, resulting in consistent and the predictable infective pattern of infection in the mice. It has been demonstrated that the most usual outcome of *T. congolense* infection in the outbred mouse is death, even with the most "resistant" mice (Pinder, 1984) or the most chronic strain of the trypanosome (Barrance and Hudson, 1986). Although the level of parasitaemias following the first peak were indistinguishable between the different dosage groups, as shown in Section 4.3.2.1, the prepatent period and the onset of mortality in the groups may have been influenced by the infective dose levels as these events occurred earlier in the higher dosage groups. This observation agrees strongly with the results obtained by Roelants and Pinder (1987) in CFLP mice. As, at least 50% of the mice survived in Experiment 4.6 till the end of observation (i.e. 30 DAI), it was still possible to investigate the responses to conjoint *T. congolense* and *H. polygyrus* in them (see Chapters 8 ,9 and 10).

The drop in PCV associated with the onset of parasitaemia due to *T. congolense* (Section 4.3.2) is consistent with the observation that anaemia is the most common feature of African trypanosomosis in experimental and naturally infected animals (Anosa, 1983a; Igbokwe, 1989).

It is known that animals infected with trypanosomes show hepatomegaly and splenomegaly (Losos and Ikede, 1972) and in the mouse the increase in the size of these organs is several fold. The slight increase in the live weight of mice infected with *T. congolense* above that of the uninfected group (Figure 4.7b) might well have resulted from this increase in the size and weight of the liver and the spleen.

Although antibody may have a role to play in the control of infection due to *T. congolense* (Mitchell and Pearson, 1986; Roelants and Pinder, 1987), the progressive antibody response seen in all the groups of infected mice (Section

4.3.1.5) did not abate the parasitaemia in these outbred mice. Musoke *et al.* (1981) demonstrated that, in cattle, raised IgM concentrations were correlated with specific anti-trypanosome antibody rather than a total non-specific response. However, since the ELISA used in this study was based on antigen obtained from whole trypanosome sonicated extract and not on variant surface glycoprotein alone, the observed responses might have well related mostly to responses to internal antigens released following the death of trypanosomes, thus making it difficult to be correlated with the ability to control parasitaemia. Although antibody is not the sole factor contributing to murine resistance to African trypanosomes, the ability of C57B1 mice to successfully survive *T. congolense* infection for longer periods compared to the other strains of mice, has been attributed largely to their superior humoral response (MacAskill *et al.*, 1983). If so, the concentration of VSG-specific antibodies in the mice in the present study may have been inadequate to cope with the trypanosomosis, so that that many of them succumbed to the infection by 30 DAI with dose-related increasing mortality.

In conclusion, since the conditions which gave chronic and consistent infections with the bloodstream forms were much easier to reproduce than with the culture-derived metacyclics, subsequent conjoint infections of TO mice involving *T. congolense* were conducted with stabilated bloodstream forms (see chapters 8, 9 and 10).

CHAPTER FIVE

PRIMARY INFECTION OF FEMALE OUTBRED MICE WITH DIFFERING INTENSITIES OF *HELIGMOSOMOIDES POLYGYRUS* INFECTIVE LARVAE

5.1 INTRODUCTION

Heligmosomoides polygyrus has been studied in a variety of laboratory mouse strains including outbred strains such as LACA, MF1 and CFLP (Ey, Prowse and Jenkin, 1981; Behnke *et al.*, 1983; Williams and Behnke, 1983; Keymer and Hiorns, 1986a; Robinson, Wahid, Behnke and Gilbert, 1989). Although it is generally agreed that *H. polygyrus* is a long-lived parasite in the mouse, its survival pattern in the host is known to be determined mainly by the strain of the host and the intensity of infection (Prowse, Mitchell, Ey and Jenkin 1979; Dobson, Sitepu and Brindley, 1985). There are, however, no reports on the dynamics of heligmosomoidosis in TO outbred mice. The experiments reported in this chapter were designed to examine the effects of varying intensities of primary single infections with L_3 of *H. polygyrus* on the resultant worm population and the host's responses to such infections. The results of this study provided the base-line for the levels of *H. polygyrus* used in subsequent studies described in this thesis.

5.2 MATERIALS AND METHODS

5.2.2 Experimental Design and Procedures

Five groups, each of eight mice, were infected orally with 0, 60, 125, 250 and 500 L_3 respectively. Packed cell volume (Section 3.4.2) and live weight of each mouse were monitored weekly and the EPG (Section 3.2.4) was determined daily 30 days after infection (DAI) when all mice were killed. The total worm burden and *in vitro* fecundity of the female worms were determined post mortem as described in Sections 3.2.5. Acid-pepsin digestion was carried out on the small intestines in an attempt to recover immature parasites (Section 3.2.7). Recovered worms were sexed and measured as described in detail under Section 3.2.6.

To determine the influence of the worm burden on the size of the spleens of the mice at 30 DAI, the weight of the organ from each mouse was obtained and expressed as a percentage of the body weight. The antibody response was measured by an ELISA (see Section 3.7) on plasma samples collected from each mouse.

5.3 RESULTS

Eggs appeared in the faeces of mice given 500 L_3 at 9 DAI and on 10 DAI for the other groups of infected mice. Egg counts rose rapidly until about 13 DAI and then rose slowly and irregularly to first peak counts of 29,000, 43,000, 79,500 and 115,000 from mice infected with 60, 125, 250 and 500 L_3 respectively between 24 and 25 DAI (Figure 5.1). The mean egg output was directly proportional to the infective dose (r=0.9811, P<0.05). The changes in the PCV were minimal in all groups of experimental animals except for those infected with 500 L_3, whose mean PCV dropped significantly by 14 DAI when compared to control (U=6.5, P<0.01) but recovered by 28 DAI (Figure 5.2a). All groups of mice generally gained weight to a similar extent throughout the period of observation (Figure 5.2b).

No immature worms were recovered 30 DAI following acid-pepsin digestion. The adult parasite ratio was consistently female biased. The worm burden showed a positive and highly significant correlation (r=0.93, P<0.01) with the infective dose but the establishment rates were variable (Table 5.1). The mean length of male worms varied between 7.0 and 8.1 mm while females measured from 17.6 to 20.7mm (Table 5.2). The length of female worms correlated negatively with the dose of infection (r=0.509, P<0.01). The *in vitro* egg production of female worms over a 24 h period was similar for each group (Table 5.3). The splenic weights increased with increasing infective dose of *H. polygyrus*, contributing to 0.5 to 0.78% of the total live weight of infected mice (Figure 5.3).

The protein content of NOG, CTAB and homogenized adult worm extracts (Sections 3.6.1 and 3.6.2) were 0.055, 0.208, and 2.56mg/ml respectively. Because of high protein content, only the homogenized adult worm extract was used in the serological studies reported in this chapter and in subsequent studies. Based on the optimum values obtained from the titration curves (Figures 5.4a, b and c), protein concentration of 5μg/ml, the conjugate dilution of 1:2000, and 1:50 dilution of the test serum were selected for ELISA.

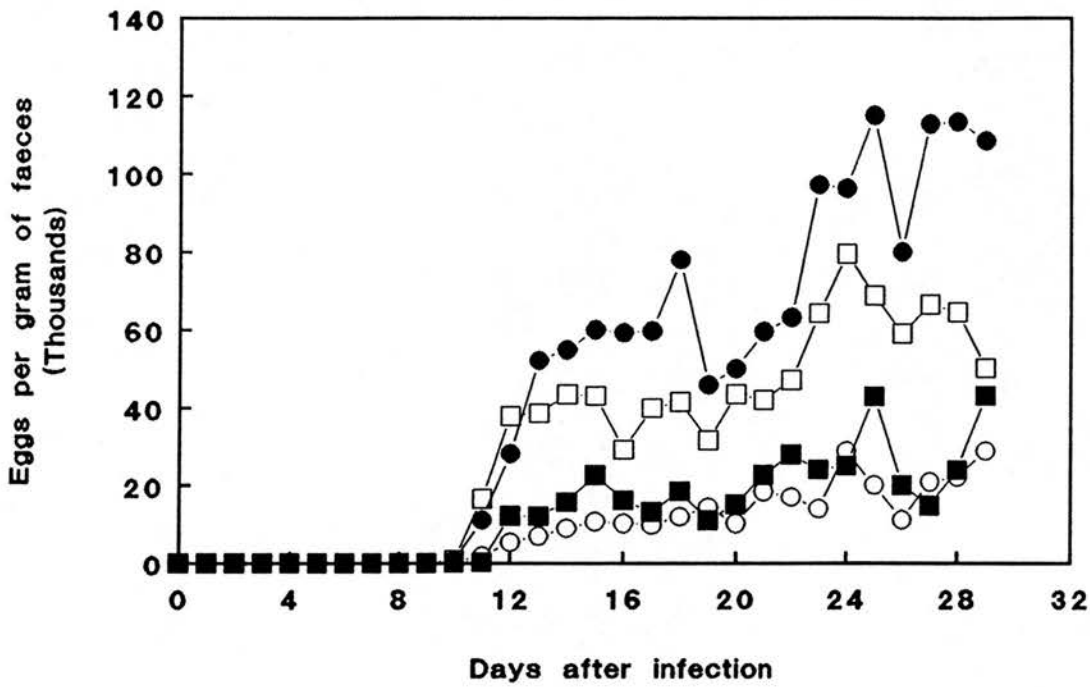

FIGURE 5.1 The faecal worm egg count of mice infected with 60 L_3 (○), 125 L_3 (□), 250 L_3 (■) or 500 L_3 (●) of *H. polygyrus*

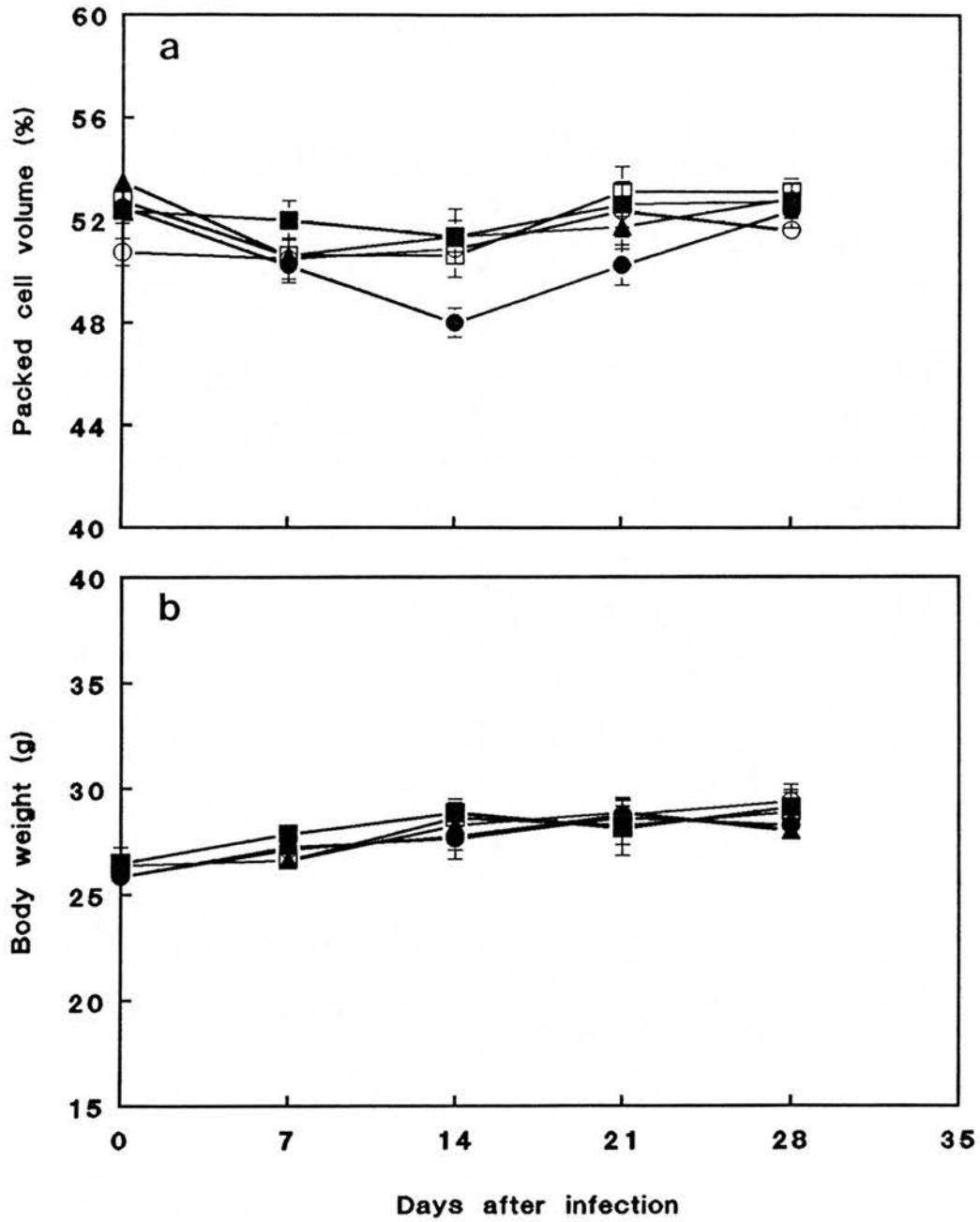

FIGURE 5.2 The mean (±SEM) weekly PCV (a) and live weight (b) of mice infected with 60 L_3 (○), 125 L_3 (□), 250 L_3 (□) or 500 L_3 (●) or not infected (▲) with *H. polygyrus*.

TABLE 5.1 The sex ratio (male:female), mean burden and establishment rate of *H. polygyrus* in experimentally infected female TO mice at 30 days after infection

Inoculum size	Mean worm burden (SEM) Male	Female	Total	Establish-ment (%)	M:F*
60	13.8(1.2)	23.8 (2.0)	37.5 (2.7)	62.5(4.4)	0.58
125	20.0(3.0)	32.6 (4.1)	52.6 (6.6)	42.1(5.3)	0.61
250	64.1(6.8)	92.0 (9.5)	156.1 (15.2)	62.5(6.1)	0.70
500	112.1(9.2)	165.9(12.4)	278.0 (19.0)	55.6(3.8)	0.67

* Male:Female ratio.

TABLE 5.2 Influence of inoculum size on the length of adult *Heligmosomoides polygyrus* recovered from TO mice at 30 DAI

Inoculum size (L_3)	Mean length of worm in millimetres (SEM) Male	Female
60	8.1 (0.1)	20.7 (0.5)
125	7.0 (0.1)	18.4 (0.3)
250	7.4 (0.1)	18.6 (0.4)
500	7.1 (0.1)	17.6 (0.3)

TABLE 5.3 The average number of eggs passed by female *H. polygyrus* in the different experimental groups into Hanks' BSS during the 24 hours of incubation at 37°C

Inoculum size (L_3)	Mean number of eggs/female worm in 24 h (SEM)
60	406.1 (31.7)
125	417.0 (43.0)
250	490.6 (40.3)
500	414.9 (34.3)

The ELISA technique described in Section 3.7 was used to detect antibodies against *H. polygyrus* antigens in samples of mouse plasma. Samples were examined in duplicate. ELISA values for each animal on each occasion consisted of the average of the results obtained using its serum, while the values for each group represent the means for the individual mice corrected as described in Section 3.7. There was a gradual increase in the antibody titres for the infected mice as the infection progressed (Figure 5.5). At 28 DAI, the responses of infected mice differed significantly from the uninfected control. Those infected with 60 L_3 showed a weaker response (U=12, P<0.05) but those infected with 500 L_3 showed a more significant reponse (U=3, P<0.01) compared to the uninfected control.

5.4 DISCUSSION

In this study the faecal egg counts closely related to the adult worm burden, which was itself directly proportional to the number of *H. polygyrus* L_3 in the primary inoculum. Although other authors have considered that factors such as worm density and the amount of faecal output can affect the faecal worm egg output (Anderson and Schad, 1985), the strong correlation between the worm burden and the EPG obtained in this study and several field studies (Roberts and Swan, 1981; Fakae, 1990b) shows that faecal egg counts can provide a quick and reliable technique for monitoring trends in the burden of strongyle worms in animals. It was assumed, although not proven, that the number of eggs passed *in vitro* may reflect the fecundity of the female worm. If this be the case, since there was no dose dependent effect on the fecundity of individual worms, the level of faecal egg output was a direct reflection of the worm burden.

The absence of overt clinical signs of heligmosomoidosis in the present study, even in the highest infective dosage group, indicates that TO mice tolerate a chronic primary *H. polygyrus* infection. This is demonstrated well in the similar live weight gains in all groups of experimental animals. The slight drop in PCV in mice infected with 500 L_3 during the second week was probably due to traumatic emergence of the larger numbers of juvenile worms. Baker (1955) observed a

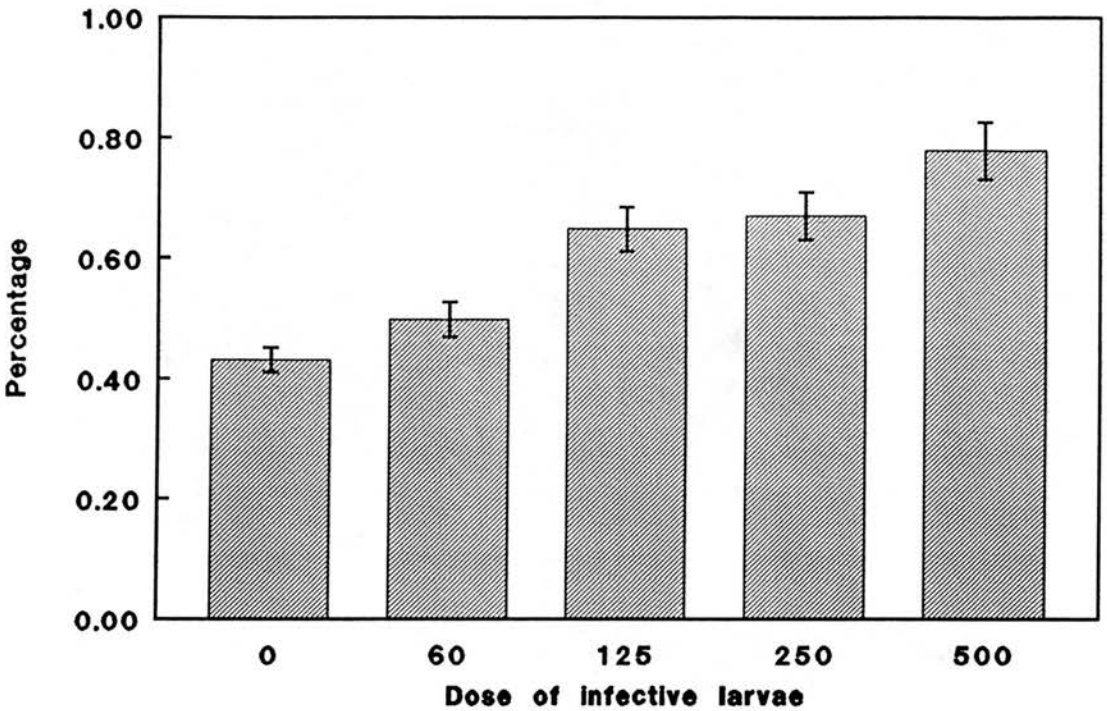

FIGURE 5.3 The mean (±SEM) spleen weight as a proportion of the body weight of mice infected with varying numbers of *H. polygyrus*

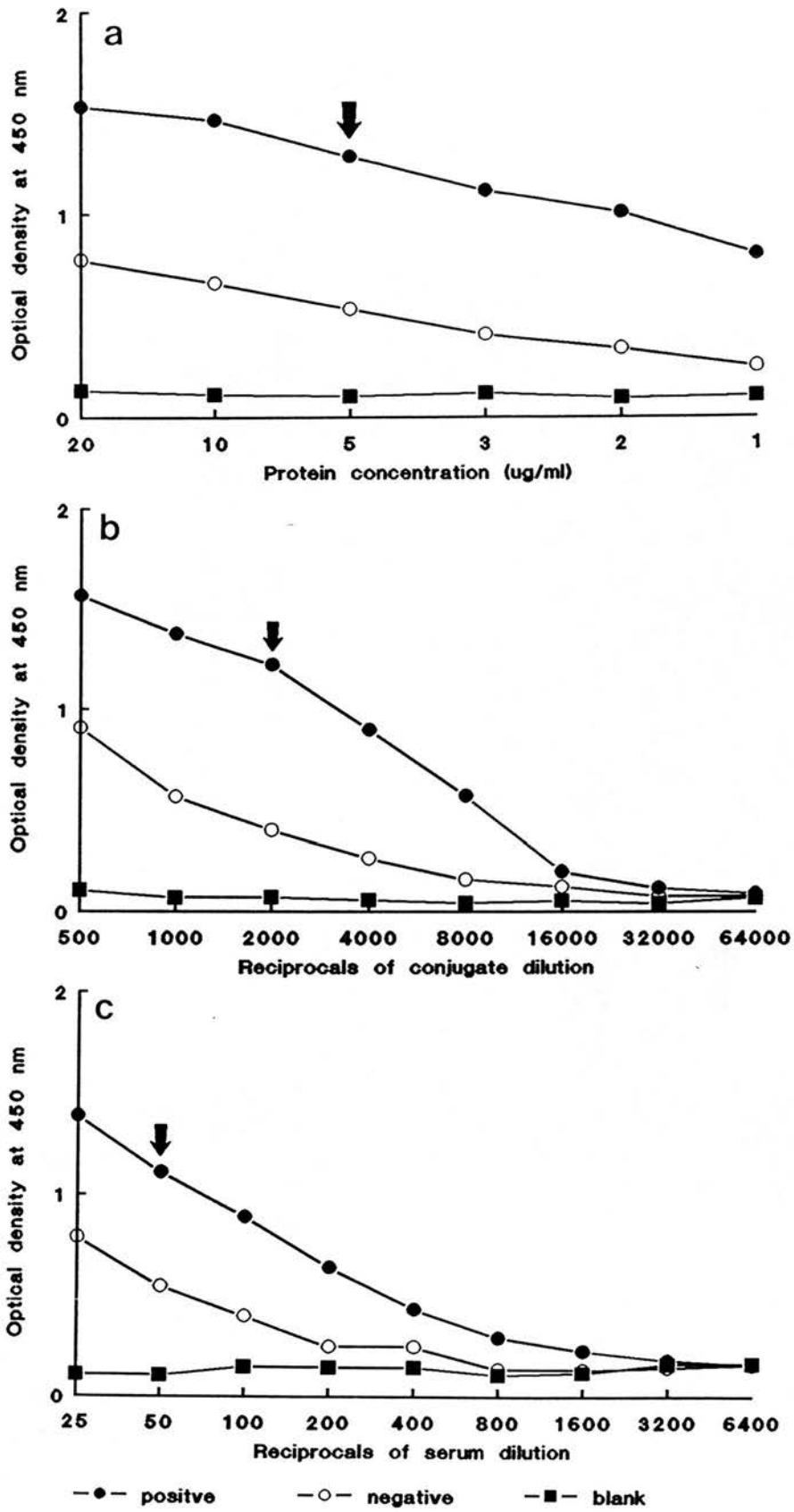

FIGURE 5.4 The results of ELISA titrations performed with known positive antisera against *H. polygyrus* and negative sera to determine the optimum (a) concentration of parasite antigenic protein, (b) enzyme conjugate (goat anti-mouse peroxidase) dilution and (c) dilutions of serum samples. The arrows indicate the optima deduced from the curves

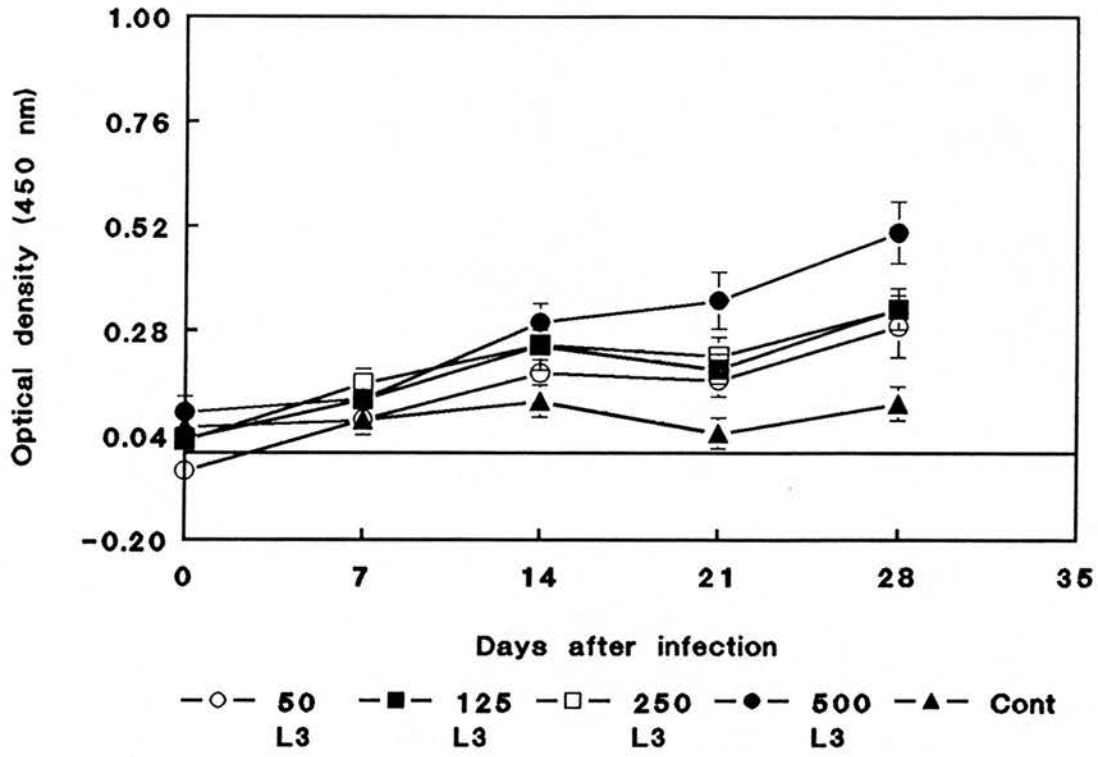

FIGURE 5.5 The ELISA values using sera from mice following different infective doses of *H. polygyrus*

marked reduction in the erythrocytic parameters, namely, PCV, red blood cell (RBC) count and haemoglobin level at the same time after infection. However, anaemia is not a major pathological manifestation of heligmosomoidosis since the blood parameters returned to the preinfection levels by about 28 days after infection in contrast to the situation with hookworms or *Haemonchus contortus* (Soulsby, 1982).

The reason for the female biased population of gastrointestinal nematodes such as observed in this study remain a matter of speculation. Sex distribution did not appear to be influenced by the level of *H. polygyrus* infection. Keymer and Hiorns (1986a) suggested that this may result from differential mortality of the male and female larvae during their passage into the intestine and development in the intestinal mucosa. It is also possible that there is a similar bias in the eggs passed by the worms.

The absence of immature worms indicates uninterrupted development of *H. polygyrus* during primary infection in the TO strain of mouse. By 30 DAI the worms had developed fully and were rather longer than reported elsewhere, where the lengths were determined between 14 and 20 DAI (Panter, 1969a; Pritchard and Behnke, 1985; Slater and Keymer 1986b; East *et al.*, 1988). In the present study, a density dependent factor appears to have affected the size of the female but not of the male worms. The differential effect on the sexes may have resulted from competitive interaction for space, having a greater impact on the comparatively larger female worms.

Splenic weights obtained 30 DAI were influenced strongly by the worm burden. The enlargement of the spleen and other lymphoid organs has been associated with the presence of adult worms in the intestine of mice undergoing a primary infection and the hypertrophy has been suggested to reflect the immunological activity within these organs (Humphery and White, 1971; Parker and Inchley, 1990a). However, splenectomy does not affect the course of *H. polygyrus* infection in mice (Baker, 1955), probably because splenic involvement

is thought to be secondary to the capacity of the mesenteric lymph nodes to handle the products of infection such as toxins and antigens (Ali and Behnke, 1985).

The host's antibody response during primary infection was shown to be ineffective as a defence mechanism against *H. polygyrus*, as resistance could not be demonstrated in animals given pools of primary infection serum taken 10 and 17 weeks after infection (Williams and Behnke, 1983). Although only the total serum IgG was assayed in this study, analysis of the profile of Ig isotypes and subclasses associated with *H. polygyrus* infections in mice indicates that the rise in serum Igs is mainly attributable to IgG1 (Williams and Behnke, 1983). The relatively high response in mice infected with 500 L_3 may be related to the greater antigenic load arising from the higher worm burden in this group, but this did not affect the worm burden since a similar proportion became established in each experimental group. Adult *H. polygyrus* are believed to actively suppress their host's ability to respond to homologous antigens in the gut (Cayzer and Dobson, 1983; Pritchard and Behnke, 1985) and may also increase IgG catabolism in infected mice (Brown, Crandall and Crandall, 1976), so impairing any protective immunity. In this way, *H. polygyrus*, can evade the host's immune system and give rise to a chronic primary infection in the mice (reviewed by Behnke, 1987; 1990).

In conclusion, this study has shown that the female TO mice can tolerate quite high numbers of *H. polygyrus* for a considerable time, making it a useful model for studies intended to simulate chronic infections of gastro-intestinal nematodes of ruminants. Accordingly, it was decided that mice be infected orally with a standard dose of 500 L_3 of *H. polygyrus* in later studies.

CHAPTER SIX

ANTHELMINTIC TREATMENT OF PRIMARY LARVAL AND ADULT *HELIGMOSOMOIDES POLYGYRUS* INFECTIONS IN FEMALE 'TO' MICE

6.1 INTRODUCTION

Various preparations of pyrantel, given orally are widely reported to terminate adult *H. polygyrus* infection in mice (Behnke and Wakelin, 1977; Jacobson *et al.*, 1982; Kerboeuf and Jolivet, 1984) whereas ivermectin is known to have very high efficacy against the tissue resident larval stages as well as against adult *H. polygyrus* (Sayles and Jacobson, 1983). The efficacy of these anthelmintics may be affected by the dose, route of administration and the anthelmintic-resistance status of the parasite strain and also by the strain of the host Wahid, Behnke and Conway (1989a).

There was currently no information on the efficacy of pyrantel or ivermectin against *H. polygyrus* in TO mice Since treatment of different stages of *H. polygyrus* was required in some later experimental manipulations, it was necessary to investigate the efficacy of these anthelmintics.

6.2 MATERIALS AND METHODS

Eighty female TO mice were assigned to 10 groups of eight mice each. They were orally infected with the desired infective doses of *H. polygyrus* as previously described in Sections 3.1.1 - 3.1.2. Infected mice were either treated with pyrantel embonate at a dose rate of 100mg/kg live weight (see Section 3.1.4.1) 12 DAI or ivermectin at a dose rate of 20mg/kg live weight (see Section 3.1.4.2) 6 DAI or left as untreated controls (Table 6.1). All the mice were killed 15 DAI and the total worm burden was determined post mortem as described in Section 3.2.5.

6.3 RESULTS

The mean establishment rates of the various infective doses of *H. polygyrus* and the efficacy of the anthelmintic treatments are presented in Table 6.2.

TABLE 6.1 Experimental design for infection of TO mice with *Heligmosoides polygyrus* and subsequent treatment with either ivermectin or pyrantel

Group	Primary infection	Anthelmintic treatment		*Post mortem*
		Ivermectin	Pyrantel	
	Day 0	Day 6	Day 12	Day 15
I50	50 L_3	√		√
I50(c)	50 L_3			√
I500	500 L_3	√		√
I500(c)	500 L_3			√
P50	50 L_3		√	√
P50(c)	50 L_3			√
P250	250 L_3		√	√
P250(c)	250 L_3			√
P500	500 L_3		√	√
P500(c)	500 L_3			√

TABLE 6.2 Efficacy of ivermectin and pyrantel embonate against stages of *H. polygyrus* in female TO mice.

Dose (L_3)	Mean no. of worms in control groups	Establish-ment(%)	Mean no. of worms in treated groups	Anthelmintic	Efficacy (%)
50	31.3	62.5	0	Ivermectin	100.0
500	262.6	52.6	0	Ivermectin	100.0
50	33.0	66.0	0	Pyrantel	100.0
250	140.1	56.1	0	Pyrantel	100.0
500	329.5	65.9	1.9	Pyrantel	99.4

6.4 DISCUSSION

Although doses of pyrantel as high as 175mg/kg had been used to remove adult *H. polygyrus* (Scott, 1988), this study corroborates and extends to TO mice the earlier reports by Behnke and Wakelin (1977) and Jacobson *et al.* (1982) that the adult stages of *H. polygyrus* can be readily removed from the intestine of mice by a single treatment with pyrantel at a dose rate of 100mg/kg. This anthelmintic, however, could not terminate larval infections (Behnke and Wakelin, 1977; Behnke and Robinson, 1985).

Ivermectin at 20mg/kg was found to be totally effective against larval infections of *H. polygyrus* in TO mice. However, when Wahid *et al.* (1989a) investigated the variables that could influence the efficacy of ivermectin against six day old *H. polygyrus* in five mouse strains (CFLP, NIH, C57BL10, BALB/C and CBA), they found that there was significant mouse-strain variation in the drug's efficacy. NIH mice, for instance, required treatment with higher doses than CFLP mice to bring about a comparable level of larvicidal activity. Wahid and Behnke (1992) have recently shown that recovery of the developing stages was reduced by over 70 % by 24 hours after treatment and as shown by measuring the worms, the remaining L_3 and L_4 stages of *H. polygyrus* were prevented from developing further. They concluded that larval stages of *H. polygyrus* were probably killed within 24 hours of treatment with ivermectin although the parasite burdens declined over a longer period lasting several days.

It was of particular importance that the oral route of administration of ivermectin effectively terminated all larval infections, irrespective of the primary infective dose of *H. polygyrus*. Ivermectin administered subcutaneously is known to show residual activity for 7-20 days, probably due to slow drug release from the site of injection and the subsequent long-lasting plasma level (Campbell, 1985; Lo, Fink, Williams and Blodinger, 1985), but only 2-4 days when administered orally (Wahid *et al.*, 1989a). Since the orally administered drug is cleared from the host system within a few days, subsequent new experimental infections, which were

always administered more than 20 days after treatment, were not interfered with (see Chapter 7).

Because of the efficacy of pyrantel embonate and ivermectin against adult and larval *H. polygyrus* respectively, it was decided that the chosen doses of these anthelmintics would be appropriate for use in later studies.

CHAPTER SEVEN

EFFECT OF DOSE AND DURATION ON AN INITIAL *HELIGMOSOMOIDES POLYGYRUS* INFECTION ON THE LEVEL OF PROTECTION AFFORDED AGAINST HOMOLOGOUS CHALLENGE IN MICE

7.1 INTRODUCTION

Several immunization procedures have been devised that render susceptible mice resistant to homologous challenge. Some workers have reported significant protection by inoculating mice with *H. polygyrus* L_3 intraperitoneally (Chaicumpa, Prowse, Ey and Jenkin, 1977; Robinson, Behnke and Williams, 1988), intravenously (Chaicumpa *et al.*, 1977) or subcutaneously (Rubin, Lueker, Flom and Anderson, 1971). However, oral administration of an immunizing infection, has been reported to be a better way to stimulate resistance (Jones, 1974), with immunity lasting from four to 12 months (Chaicumpa *et al.*, 1977; Lueker and Hepler, 1975). Oral primary infections with L_3 of *H. polygyrus* which had been exposed to 25 Krad gamma irradiation from a Cobalt 60 source have also been reported to protect mice from challenge infection (Behnke, Parish and Hagan, 1980; Hagan, Behnke and Parish, 1981; Robinson *et al.*, 1988). However, some of the irradiated larvae used in the priming infections persisted as arrested larvae (Ali and Behnke 1985).

One commonly employed method of oral immunization has been to administer two or three divided doses of *H. polygyrus* L_3 followed by anthelmintic treatment to remove the adults (Van Zandt, 1961; Behnke and Wakelin, 1977). However, it has been reported that better protection against homologous challenge infections was obtained when the priming infections were terminated at the larval rather than at the adult stage (Behnke and Robinson, 1985; Enriquez, Cypess and Wassom, 1988; Robinson *et al.*, 1988). Furthermore, the dose and duration of the immunizing infection are known to affect the degree of resistance to reinfection with *H. polygyrus* (Enriquez, Cypess and Wassom, 1988) and the effectiveness of the immunizing regimes also depends on the strain of mouse. While mice of some strain require several sensitizing infections, others need only one previous infection, terminated at an appropriate stage to develop strong resistance to reinfection (Van Zandt, 1961; Cypess and Zidian, 1975; Behnke and Wakelin, 1977; Mitchell and Prowse, 1979; Prowse, Mitchell, Ey and

Jenkin, 1979; Wahid and Behnke, 1992). The status of TO mice in this regard has not previously been investigated.

The aims of the experiments in this chapter were (1) to investigate the effect of the intensity and the duration of a primary immunizing infection on the level of protection attainable; (2) to determine a suitable procedure for immunizing TO mice against homologous challenge for use in the studies on conjoint *H. polygyrus* and *T. congolense* infections and (3) to examine the changes in the peripheral leucocyte population, gastrointestinal histology and humoral responses in TO mice associated with homologous *H. polygyrus* challenge infection.

7.2 MATERIALS AND METHODS

7.2.1 Immunization by Termination of Primary Adult *H. polygyrus* Infection (Experiment 7.1)

Forty mice were divided into five groups of eight mice each. They were either not infected or orally infected with varying numbers of *H. polygyrus* L_3. All mice were then treated with pyrantel embonate (100mg/kg body weight),12 DAI and challenged with 500 L_3 of *H. polygyrus* 8 days after anthelmintic treatment (Table 7.1) All the mice were killed 35 days after the start of the experiment and their worm burdens and *in vitro* fecundity of worms from the various experimental groups were determined. The percentage protection was calculated with reference to the total worm burden in the group which did not receive any immunizing infection.

7.2.2 Immunization by Termination of Larval *H. polygyrus* Infection (Experiment 7.2)

One hundred female TO mice were randomly selected and placed in 10 groups of 10 mice each. Except for the controls which didn't receive any immunizing infection, the mice were either infected with 50 L_3 or 500 L_3 of *H. polygyrus*. Infections were terminated with ivermectin (20mg/Kg) at various days after infection. Control mice were also treated. Challenge infections of 500 L_3 were then given to all the mice, except the uninfected controls, approximately three

weeks after the anthelmintic treatment (Table 7.2), by which time the ivermectin would have been metabolised and eliminated by the host (Wahid *et al.*, 1989a).

All the animals were killed and bled out from the heart 20 days after challenge infection. Blood smears were prepared from each mouse and stained with Giemsa for a differential leucocyte count. The sera obtained from individual mice were preserved and used in ELISA studies with *H. polygyrus* antigens.

The small intestines of two mice from each group were recovered and examined grossly. Histological sections of normal, infected and primed mice were prepared, and stained by H&E and PAS reaction to determine the histological changes in the intestines.

The worms were extracted from the intestines of each of the remaining mice, sexed and counted (see Chapter 3) Protection attributable to the different immunization regimes were calculated from the total number of worms recovered from the infected control group.

TABLE 7.1 Experimental design for immunization of TO mice against challenge *H. polygyrus* infection by abbreviation of adult infection (Experiment 7.1)

Group	Primary infection Day 0	Anthelmintic treatment Day 12	Challenge infection Day 20	Post mortem Day 35
HA50	$50L_3$	√	√	√
HA250	$250L_3$	√	√	√
HA500	$500L_3$	√	√	√
HA0	$0L_3$	√	√	√

TABLE 7.2 Experimental design for immunization of TO mice with different levels of abbreviated larval infections (Experiment 7.2)

Groups	Immunizing infection (L_3)	Day of treatment after infection	Challenge infection
HL50/1	50	1	√
HL500/1	500	1	√
HL50/2	50	2	√
HL500/2	500	2	√
HL50/3	50	3	√
HL500/3	500	3	√
HL50/6	50	6	√
HL500/6	500	6	√
HL0	0	6	√
C	0	6	-

7.3 RESULTS

7.3.1 Stimulation of Immunity to *H. polygyrus* by Termination of Adult Infection (Experiment 7.1)

The unstimulated control group harboured 360.5 (+12.9) worms. Protection against homologous challenge was dircctly but weakly related (r=0.225, P>0.05, n=24) to the immunizing dose of *H. polygyrus* (Figure 7.1). There was marked depression of in vitro egg production by the worms recovered from immunized mice, irrespective of the infective dose (Figure 7.2).

7.3.2 Stimulation of Immunity to *H. polygyrus* by Termination of a Larval Infection (Experiment 7.2)

Figure 7.3 shows the percentage protection attributable to an immunizing infection with 50 or 500 L_3 of *H. polygyrus* and the effect of the duration of that infection. There was no difference between the protection obtained from infections of 6 day duration initiated with 50 or 500 L_3 (U=23, P=0.898). However, although infections with 500 L_3 and of 1, 2, or 3-day duration produced higher protection

against a homologous challenge than those induced by 50 L_3 of the same duration only those of 3-day duration were statistically significant (U=3.2, P<0.01).

Due to logistic problems, only worms pooled from the group immunized with 500 L_3 and the control were measured. Male and female worms from the immune animals were significantly shorter (Figure 7.4) than their counterparts from the uninfected control mice ($F_{1,38}$=338.0, P<0.001; $F_{1,38}$=440.056, P<0.001 respectively).

The proportion of eosinophils generally increased in the peripheral blood with the duration of the immunizing infection, being significantly higher in the mice immunized with 500 L_3 than in those which had received 50 L_3, especially of 6-day duration (U=6.5, P=0.018). The eosinophil levels were similar in both infected and uninfected controls (Figure 7.5a). Lymphocyte proportions in infected mice were markedly depressed (Figure 7.5b), but the reverse was true for the neutrophils (Figure 7.5c). Monocyte levels were rather variable in all the immunized groups and with the different duration of immunizing infection (Figure 7.5d).

The antibody titres in the groups immunized with 50 L_3 were directly proportional to the duration of the immunizing infection (r=0.984, P=0.0158). The antibody titres obtained by immunizing with 500 L_3 also increased proportionately with the duration of infection (r=0.958, P=0.0416) but declined to a level similar to that elicited by 6 day old 50 L_3 immunizing infection.. There was a minimal antibody activity in the serum from the unimmunized infected control (Figure 7.6).

The small intestines of unprimed mice carrying *H. polygyrus* infection were generally swollen and translucent (Plate 7.1a,b). In primed mice, the small intestine contained several white parasitic nodules (granulomata) which bulged out on the serosal surface (Plate 7.1c). In the infected mice, there was hyperplasia of the epithelium, elongation of intestinal glands, distortion and hypertrophy of villi and about twice as many goblet cells as in the uninfected control (Plate 7.2a-c). In

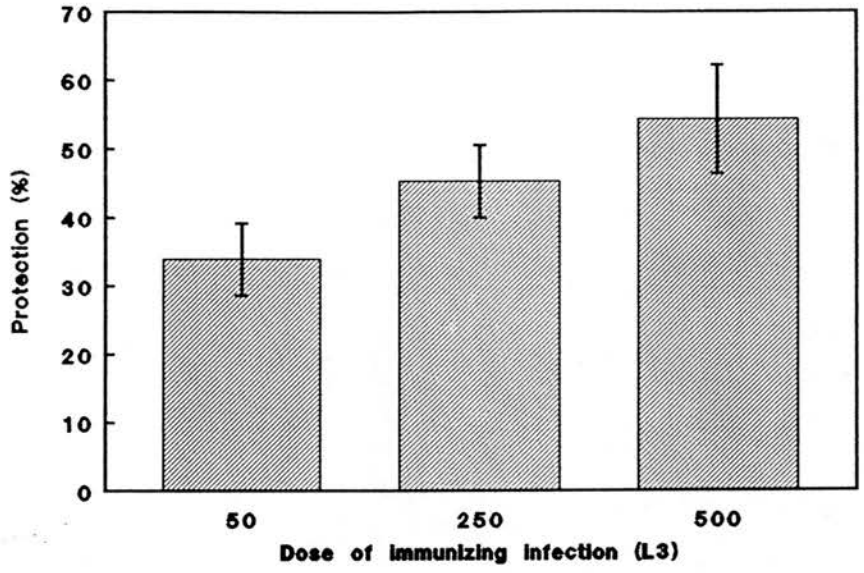

FIGURE 7.1 The mean percentage protection (±SEM) in mice immunized by termination of an adult *H. polygyrus* infection (Experiment 7.1).

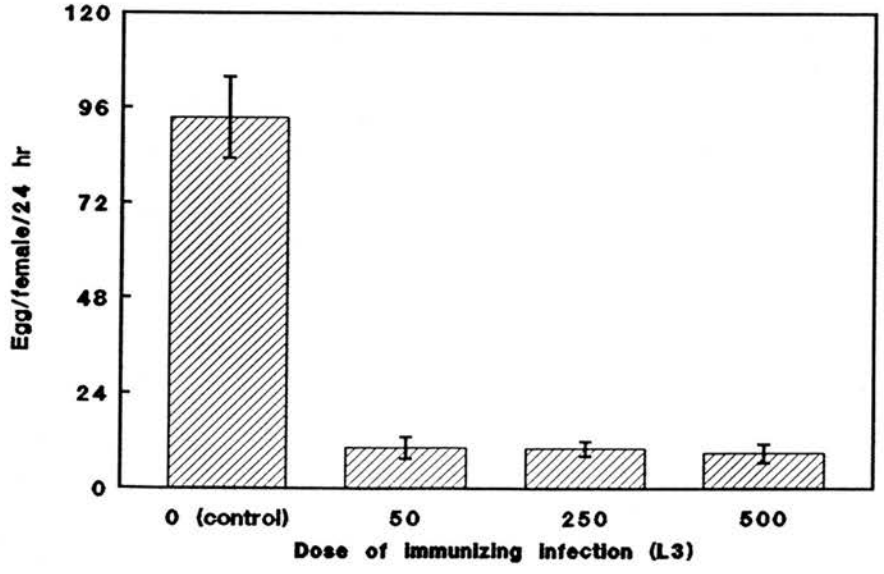

FIGURE 7.2 The mean egg production (±SEM) by female *H. polygyrus* from immunized and control mice during 24 hours *in vitro* (Experiment 7.1)

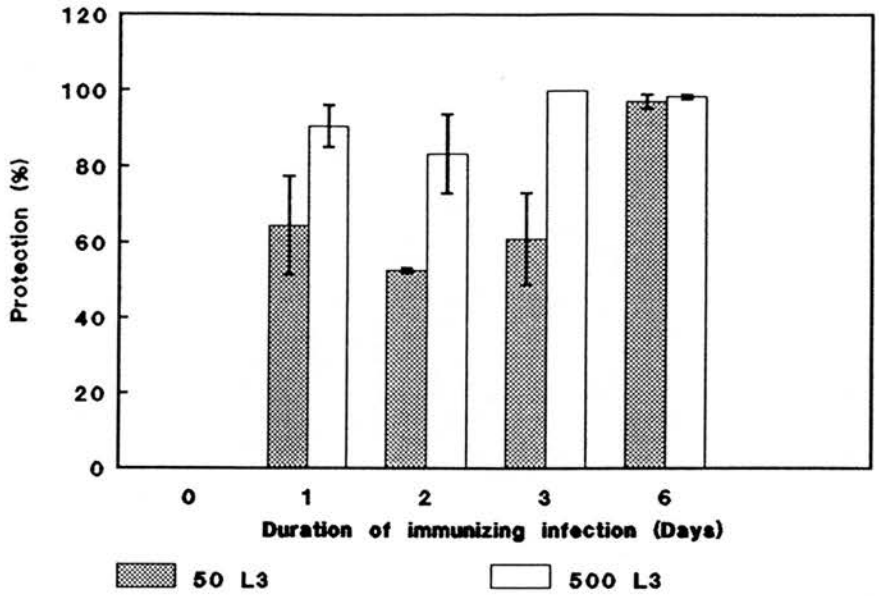

FIGURE 7.3 The mean (±SEM) percentage protection obtained from mice immunized by termination of larval *H. polygyrus* infections (Experiment 7.2).

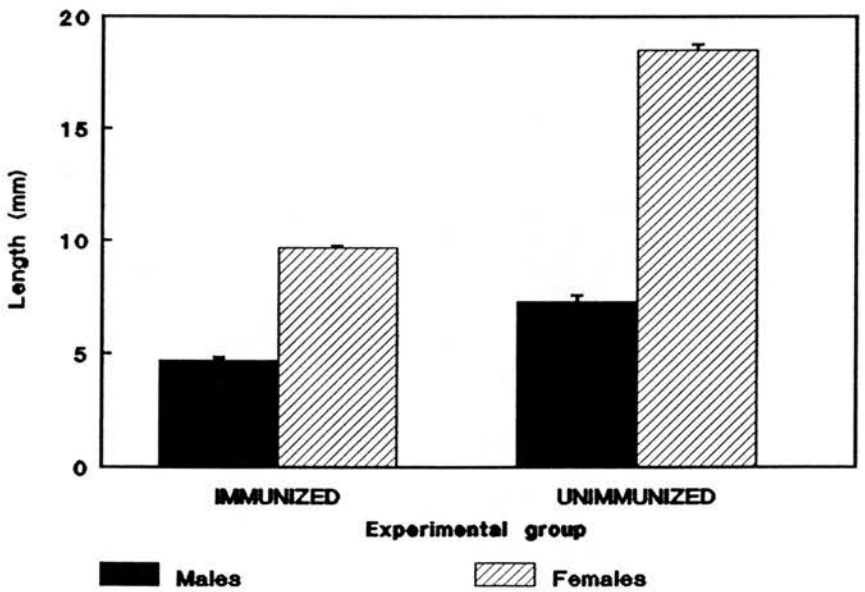

FIGURE 7.4 The mean (±SEM) lengths of male and female H. polygyrus obtained from mice immunized by termination of *H. polygyrus* larval infection and from unimmunized controls (Experiment 7.2)

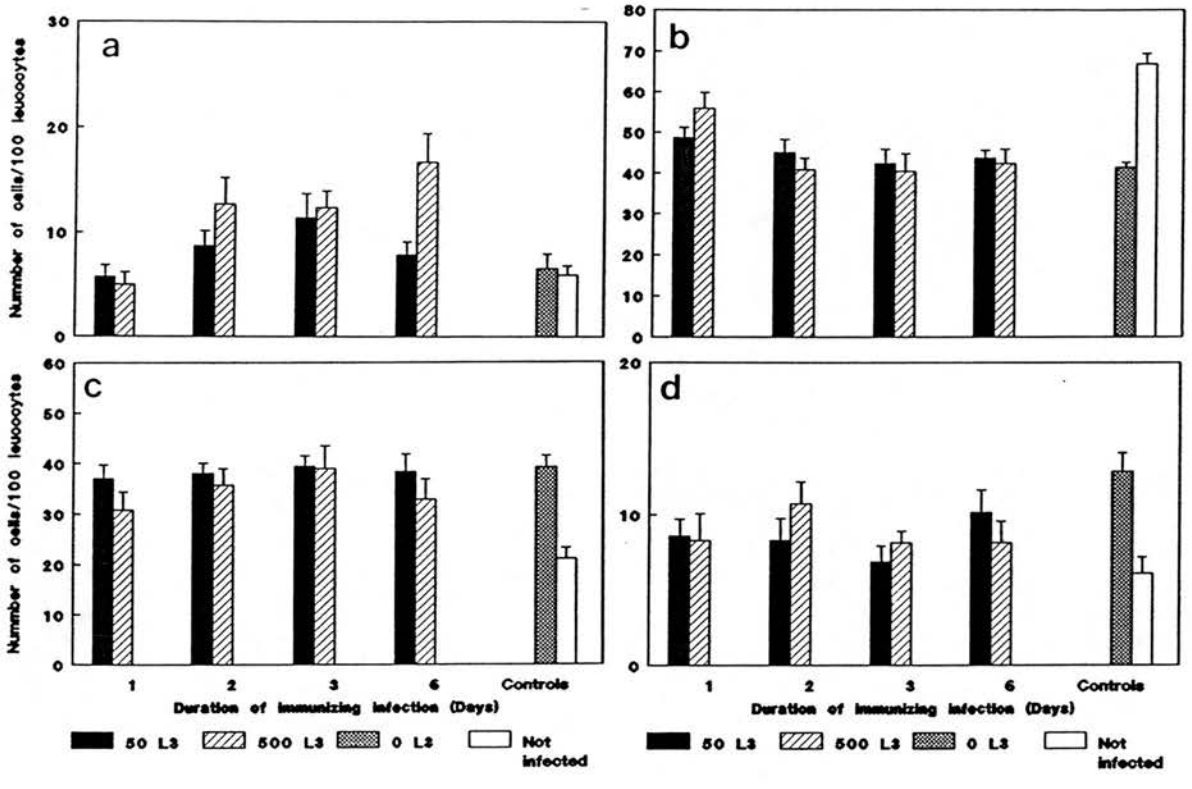

FIGURE 7.5 The mean (±SEM) (a) eosinophil, (b) lymphocyte, (c) neutrophil and (d) monocyte counts per 100 leucocytes in the peripheral blood of control mice mice and those varying doses and duration of *H. polygyrus* larval infection (Experiment 7.2)

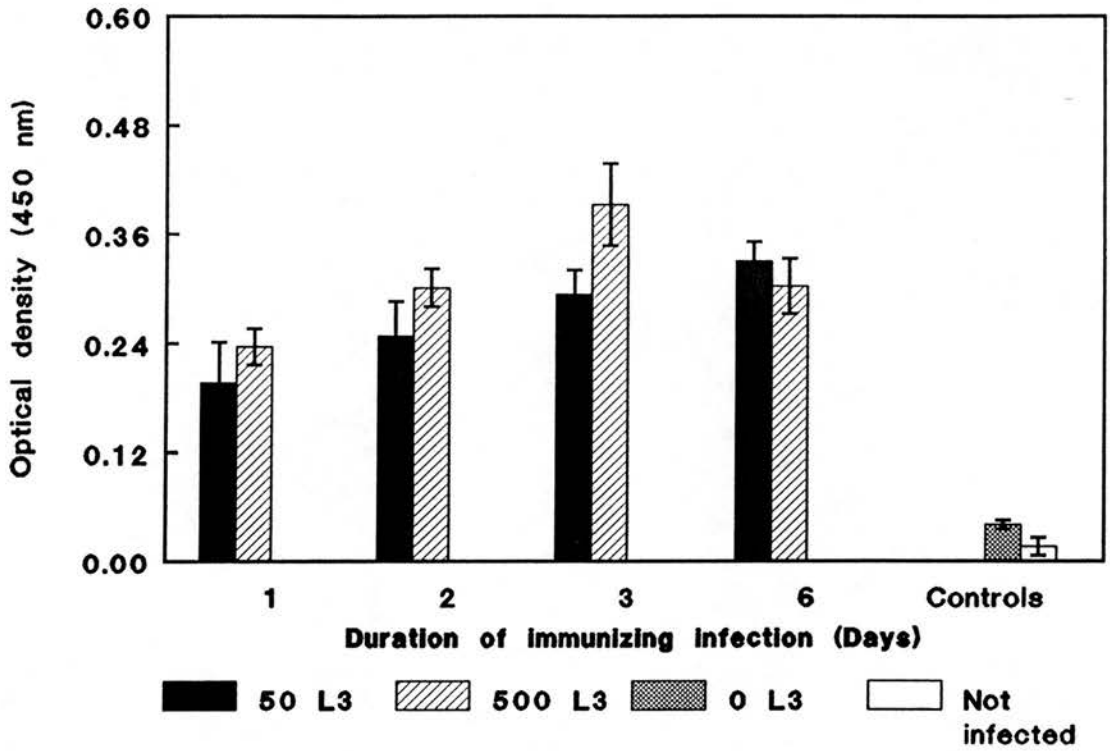

FIGURE 7.6 The mean (±SEM) ELISA serum antibody activities in control mice and those with varying doses and duration of initial *H. polygyrus* larval infection (Experiment 7.2)

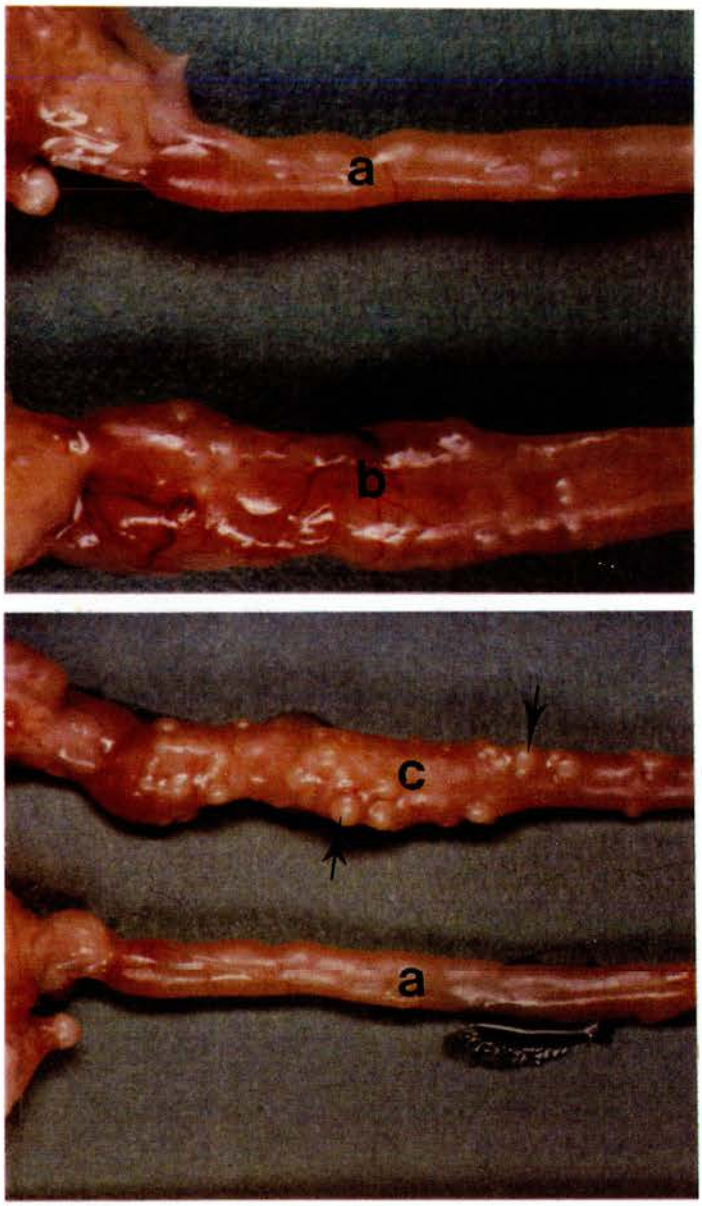

PLATE 7.1 The appearance of the small intestine (duodenum) of mice not infected (a) or infected with *H. polygyrus* (b) or primed without challenge infection (c). Arrows indicate granulomatas (Experiment 7.2)

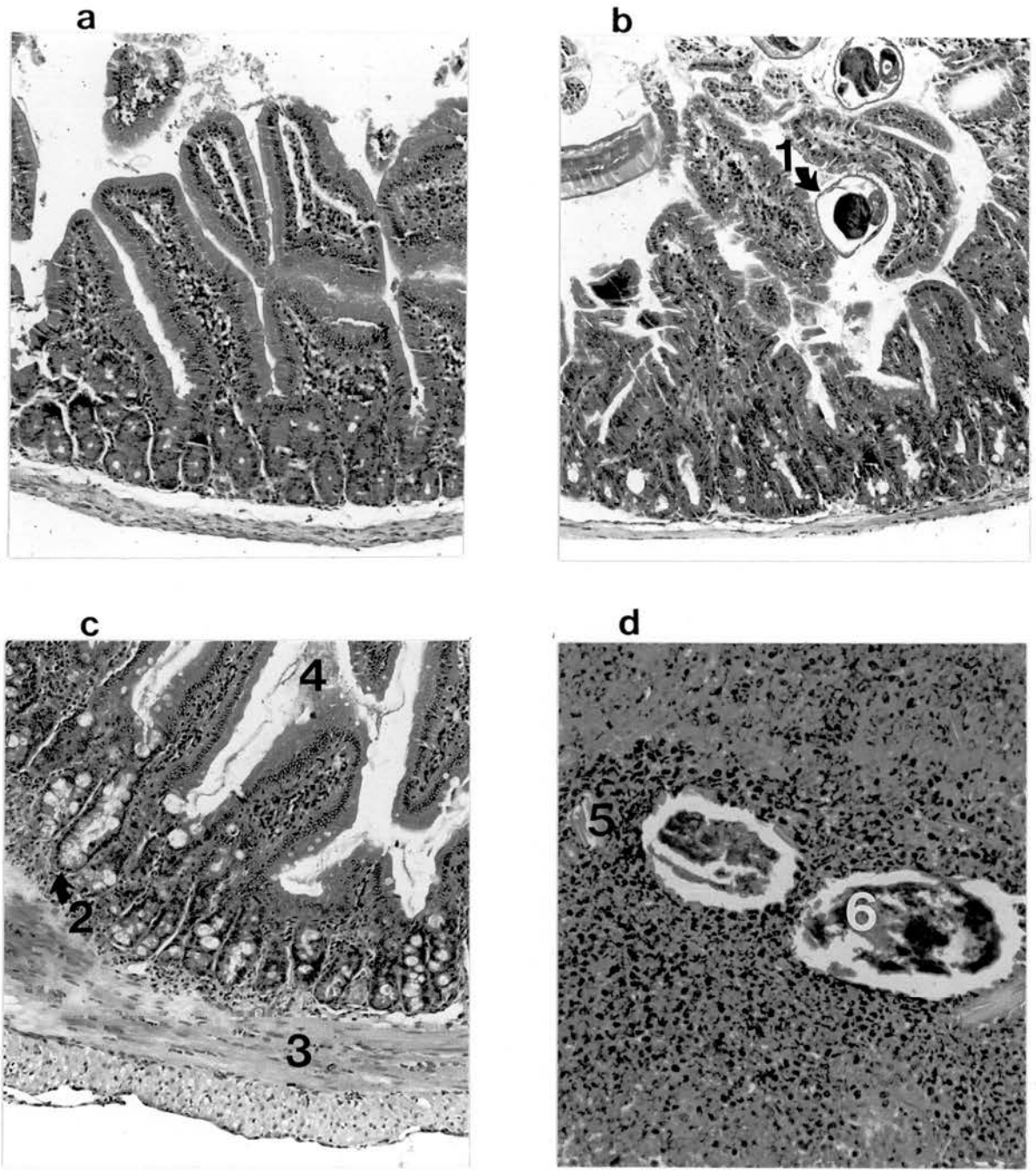

PLATE 7.2 Typical transverse histological sections, stained with haematoxylin/eosin, of the small intestines of mice not infected (a) or infected with *H. polygyrus* (b) or primed but without challenge infection (c) and granulomata from primed mice (d) (Experiment 7.2). (1=section of worm; 2=goblet cells and enlarged glands; 3=thickened *muscularis*; 4=secreted mucus; 5=remains of larval cuticle; 6=dead larva).

the primed mice, there was much thickening of the *muscularis* with extensive granulomatas associated with larvae killed within the intestinal wall (Plate 7.2d). The intestinal glands of these mice were even larger than in the infected and there was a marked intense cellular reaction involving infiltrations of neutropolymorphonuclear cells, plasma cells and numerous eosinophils. PAS reaction revealed an increase in the number and size of the goblet cells as well as the amount of mucus in the intestines of the primed animals. These reactions although present in intestines of all immunized mice, were greater in the high dose groups and particularly in the group primed with 500 L_3 for 6 days. The granulomatas contained areas of granulation tissue necrosis with several remains of the larval parasite's cuticle (Plate 7.2d).

7.4 DISCUSSION

It was clear from this study that significantly better protection was obtained against homologous *H. polygyrus* by terminating the priming infection at the larval rather than at adult stages, the level of protection being influenced by both the dose and duration of the immunizing infection (compare Figures 7.1 and 7.3). The histological changes in the gastrointestinal tract, the changes in the peripheral leucocyte population and the antibody responses suggest an immunological basis for resistance involving both the cellular and humoral components of host immunity.

It had previously been established that juvenile *H. polygyrus*, especially the L_4, are highly immunogenic and provide the essential stimuli for the expression of host-protective immunity (Van Zandt, 1961; Jacobson *et al.*, 1982, Wahid and Behnke, 1992). Reciprocal studies of sensitization involving the transfer of adults via laparotomy directly into the duodenum did not stimulate resistance, confirming that stimulation of immunity was solely attributable to larval stages (Jacobson *et al.*, 1982; Bartlett and Ball, 1974).

Wahid and Behnke (1992) have suggested that tissue resident larvae of *H. polygyrus* are immobilized within 24 hours of treatment with ivermectin, enabling

their destruction by local inflammatory and granulomatous reactions and that subsequently all the antigenic components of these stages are made available to the host's immune system. Although the larvae are sequestrated by a localized leucocytic response, it has been suggested that there is no evidence of true cyst formation around them (Sukhdeo, O'Grady and Hsu, 1984) so that there can be ready and continuous contact between the larval antigens (both somatic and cuticular) and the immune system. The proportional increase in the protection with the size and duration of the immunizing larval infection observed in the present study may be related to the amount of the essential larval antigenic materials to which the host was exposed. Even after the larvae have been killed by the anthelmintic, these materials would still be available as the larvae were trapped in the intestinal wall. This killing of the immunogenic larval stages by ivermectin within the intestinal wall may well be an important factor since it ensures the provision of the needed stage specific antigen at an appropriate location.

The results of the present study support the collective data on the role of stage specific antigens in protection against *H. polygyrus* (Van Zandt, 1961; Bartlett and Ball, 1974; Jacobson *et al.*, 1982; Wahid and Behnke, 1992). It shows that an immunizing dose of 500 L_3 will afford maximum protection to TO mice even if it is terminated as early as 3 days after infection. The superiority of larval immunization over an adult truncated infection is further demonstrated by the fact that even an infection with 50 L_3 for one day protected the mice better than the highest dose terminated at the adult stage. This despite the fact that the adult immunizing infection must have been through the larval stage - that is, there is a loss of protection between six and 12 days after infection. This may be a reflection of how the parasite survives in the host for an extended period (Behnke, 1987).

It has been shown the adult stages of this parasite have an immunosuppressive effect (Jenkins and Behnke, 1977; Behnke and Parish, 1979; Jacobson *et al.*, 1982) and it has been suggested that this may be due to secretion of some immunomodulatory factors which are capable of depressing the expression of

homologous immunity (Behnke *et al.*, 1983). It appears from this study (Experiment 7.1), that as little as 3 days of the presence of adult *H. polygyrus* is enough to reduce the protection against homologous challenge. These observations thus confirm and extend to the TO strain of mice earlier reports (Kerboeuf and Jolivet, 1984; Behnke and Robinson, 1985; Enriquez *et al.*, 1988) that infections of *H. polygyrus* terminated before the parasites became adult generate a considerably more potent immunity than those in which the infection is allowed to progress to patency.

Results of several studies suggest that antibody, thymus-dependent lymphocytes and non-lymphoid cells may all be involved in the loss of worms from immune animals (reviewed by Wakelin, 1978b). The successful passive immunization of mice by transfer of immune serum (Bartlett and Ball, 1974; Behnke and Parish, 1979) and the identification of a specific protective anti-worm IgG (Pritchard, Williams, Behnke and Lee, 1983; Williams and Behnke, 1983) confirm the role of humoral immunity in protection against homologous *H. polygyrus* challenge infection in mice. Pritchard *et al.* (1983) immunoprecipitated antigens of 17, 18 and 20 kDa with purified IgG1 from hyperimmune serum which was effective in passively transferring immunity. Wahid and Behnke (1992) recently also observed a considerably high reactivity in recognition of these antigens by sera from mice sensitized by abbreviated 4 to 6-day old infections. The positive correlation of antibody activities against the relatively crude antigenic preparation with the protection obtained in this study agrees with these earlier reports and also suggests that antibodies play an important role in resistance to challenge infections.

On the other hand, Bartlett and Ball (1974) reported that, in mice selectively depleted of thymus-derived lymphocytes, no inflammatory response occurred during *H. polygyrus* challenge and that the delayed maturation characteristic of resistance was abolished. Investigations into the role of T-cell subsets and cytokines in the regulation of helminth infections suggest that $CD4^+$

cells, especially the T_H2 cells are important for host protection during *H. polygyrus* infections (reviewed by Scott and Kaufmann, 1991; and Finkelman *et al.*, 1991). Although the exact mechanisms by which this is achieved are still unclear, there are suggestions that cytokines do regulate other non-lymphoid cells.

One prominent feature of response to gastrointestinal helminths in mammals is the accumulation of cells such as eosinophils, mast cells and globule leucocytes in the wall of the gastrointestinal tract (Miller, 1984; Rothwell, 1989). Eosinophilia, in particular, is a major and well-known host reaction to helminths, including *H. polygyrus*, infections (Jones and Rubin, 1974; Hurley and Vadas, 1983; Slater and Keymer, 1988). Hypodense eosinophils have been observed in the peripheral blood of rats in association with *T. spiralis* infection (Hamada, Watanabe and Kobayashi, 1992). These eosinophils are larger in diameter and have a more potent cytotoxic activity. Non-specific stimulation of eosinophilia in CBA/H mice which are relatively poorly responsive to *H. polygyrus* enhanced their ability to expel worms (Hurley and Vadas, 1983). In present study, eosinophilia correlated significantly with the level of protection confirming the observations of Cypess (1972) that there is an association between such peripheral blood changes and immune reactions to *H. polygyrus*. In spite of these, association of eosinophils with direct killing of the worms is still controversial.

Several studies have suggested that the larvae are not inhibited but are expelled by an immediate rather than by a delayed hypersensitivity reaction (Panter, 1969b; Jones and Rubin, 1974; Larrick, Semprevivo, Maloney and Tritschler, 1991). Although the process of expulsion of the challenge infection was not studied in the current experiments, histological observations in the small intestine of the primed mice, which showed that there were more goblet cells, hyperplasia and accumulation of eosinophils and numerous stimulated lymphoid nodules, suggest that a hostile environment awaited the incoming larva. It is thus likely that such conditions would be detrimental for development and survival of the parasite. It is also believed that a fresh intake of larvae by immune mice could

initiate an anaphylactic reaction which may prevent a large proportion of the invading larvae of *H. polygyrus* from establishing (Panter, 1969b). However, it seems that the proportion of larvae that are affected in this manner depends on the level of resistance. It is likely that in mice that are not solidly resistant, the residual larvae would be surrounded by inflammatory cells and probably killed in the inflammatory nodules, while those that survive return to the gut lumen only as stunted pre-adults (Ey, 1988). From the histological observations made in this study (Plate 7.2), it is likely that the action of mucus associated with the increased number of goblet cells, and the thickening of intestinal epithelium are physical obstacles which may also militated against the nematode's survival in the gut of immuned host.

It has been reported that the effects of resistance on the surviving population of *H. polygyrus* include retarded growth, decreased fecundity and eventually death (Panter, 1969a; Bartlett and Ball, 1974). Such effects were clearly demonstrated in this study by the reduced fecundity of worms from immunized mice and the stunting of the male and female worms (Figure 7.4).

Although Larrick *et al.* (1991) has recently demonstrated the effectiveness of immunization by subcutaneous vaccination with live post-infective larvae of *H. polygyrus*, the larval immunization procedure adopted by Wahid and Behnke (1992) and that used in the present study combine the benefit of an effective treatment to remove all existing worm burden with the production of a good protection against challenge. As a result of these studies, it was decided that immunization by abbreviated adult and larval *H. polygyrus* infections would be used to obtain medium and strong protection respectively against homologous challenge in the later experiments.

CHAPTER EIGHT

CONJOINT PRIMARY *HELIGMOSOMOIDES POLYGYRUS* AND *TRYPANOSOMA CONGOLENSE* INFECTIONS IN FEMALE 'TO' MICE

8.1 INTRODUCTION

Field experiments in goats (Griffin *et al.*, 1981a;b) and in N'Dama cattle (Kaufmann *et al.*, 1992), indicate that concurrent infection of *Trypanosoma congolense* and *H. contortus* produce greater pathological effects than single infection with each parasite. Although these studies suggested that the enhanced pathology in that heterologous interaction was due to the immunosuppression caused by the protozoan, simple additive effects of the pathology caused by the individual parasite populations may play a significant role, both causing anaemia.

Various laboratory animal models have been studied in an attempt to reveal the individual host- and parasite-related mechanisms in such interactions (see review by Christensen *et al.*, 1987). Urquhart *et al.*, (1973) reported a reduced ability to expel *Nippostrongylus brasiliensis* in mice concurrently infected with *T. brucei*. *Trichuris muris* expulsion was also reduced when mice were concurrently infected with *T. brucei* or *Plasmodium berghei* (Phillips *et al.*, 1974). In contrast, *T. spiralis*-infected mice were shown to have an enhanced resistance to *P. berghei* (Ngwenya, 1982). Although *Trichinella spiralis* impaired resistance to *T. musculi* infection in mice, *Heligmosomoides polygyrus* failed to have a significant enhancing effect on *Trypanosoma musculi* parasitaemia (Bell *et al.*, 1984a). These contrasting results show that the outcome of conjoint nematode and blood protozoa infection depends on both the host-parasite system and the relative timing of the dual infections.

It was desirable to establish a laboratory model resembling the natural host-parasite system so that the interactions between the nematodes and trypanosomes could be studied more closely. *Heligmosomoides polygyrus* infection, however, contrasts with *H. contortus* in that it does not cause profound anaemia, but its chronic survival and predilection site in the host are similar to those of *T. colubriformis* which commonly occurs with *H. contortus* under the same trypanosome challenge in the tropics (Chiejina, 1987). Experiments in this chapter examine the effects of conjoint *T. congolense* infection on the host and parasites during different stages of primary *H. polygyrus* infection in TO mice.

8.2 MATERIALS AND METHODS

8.2.2 Experimental Design and Procedures

Sixty four female TO mice were split into eight groups of eight mice each and infected with *H. polygyrus* or *T. congolense* as detailed in Table 8.1. Standard doses of *H. polygyrus* (500 L_3) and bloodstream forms of *T. congolense* (10^4) were used throughout. Groups of mice were either conjointly infected at day 0 or given *T. congolense* at day 5 or day 10 to coincide with the larval or adult stages of development of *H. polygyrus*.

The PCV (Section 3.4.2) and live weight of each mouse were determined weekly. The daily faecal egg count (Section 3.2.4) for each group of mice infected with *H. polygyrus* and the parasitaemia of each mouse infected with *T. congolense* were monitored. The experiment was terminated 30 days after *H. polygyrus* infection. The total worm burden and *in vitro* fecundity of female worms were determined post mortem as described in Section 3.2.5. The worms recovered were sexed and measured as described in detail in Section 3.2.6.

To determine the effect of the infections on the size of mouse spleen at 30 DAI, the organ from each mouse was weighed and its weight expressed as a percentage of the body weight. Specific anti-worm and anti-trypanosome antibodies were measured by ELISA (Section 3.7), using either homogenate of adult *H. polygyrus* or a *T. congolense* sonicate as antigens in the assay.

The whole experiment (Experiment 8.1) was later repeated (Experiment 8.2) so as to confirm the results found in the original study.

8.3 RESULTS

In both experiments, the faecal egg outputs rose rapidly to their highest levels between 12 and 20 DAI and then plateaued at about 100,000 EPG till the end of the experiment, irrespective of the combination of infection (Figures 8.1a,b).

TABLE 8.1 Experimental plan for the infection of female TO mice with *H. polygyrus* (HP)and *T. congolense* (TC)

Group	Days after infection (DAI) with *H. polygyrus* 0	5	10
Conjoint (day-0)	HP+TC	-	-
Conjoint (day-5)	HP	TC	-
Conjoint (day-10)	HP	-	TC
Nematode alone	HP	-	-
Trypanosome alone (day-0)	TC	-	-
Trypanosome alone (day-5)	-	TC	-
Trypanosome alone (day-10)	-	-	TC
Uninfected	-	-	-

The *T. congolense* parasitaemia in surviving mice given a dual infection were indistinguishable from control mice except in Experiment 8.1, where the mean parasitaemia in mice concurrently infected with *T. congolense* 10 days after *H. polygyrus* infection was slightly lower (Figures 8.2a,b).

All the uninfected mice and those only infected with *H. polygyrus* survived till the end of the observation. Mortality commenced earlier and was generally greater in groups with conjoint *H. polygyrus* and *T. congolense* infection than in the respective trypanosome control groups. While mortality commenced at 9-10 days after *T. congolense* infection in mice with dual infection on day 0 or day 5, it did not commence until 14 and 18 days in Experiments 8.1 and 8.2 respectively in mice infected with *T. congolense* when *H. polygyrus* infection was 10 days old. The overall mortality was greater in groups jointly infected during the larval development of *H. polygyrus* (i.e. day 0 and day 5) rather than during the adult stage (Figures 8.3a,b) especially in day-0 group (U=29, P=0.0002). The mortality of those conjointly infected on day 0 in both experiments was significantly greater than similar groups infected on day 5 (U=52.5, P=0.0047).

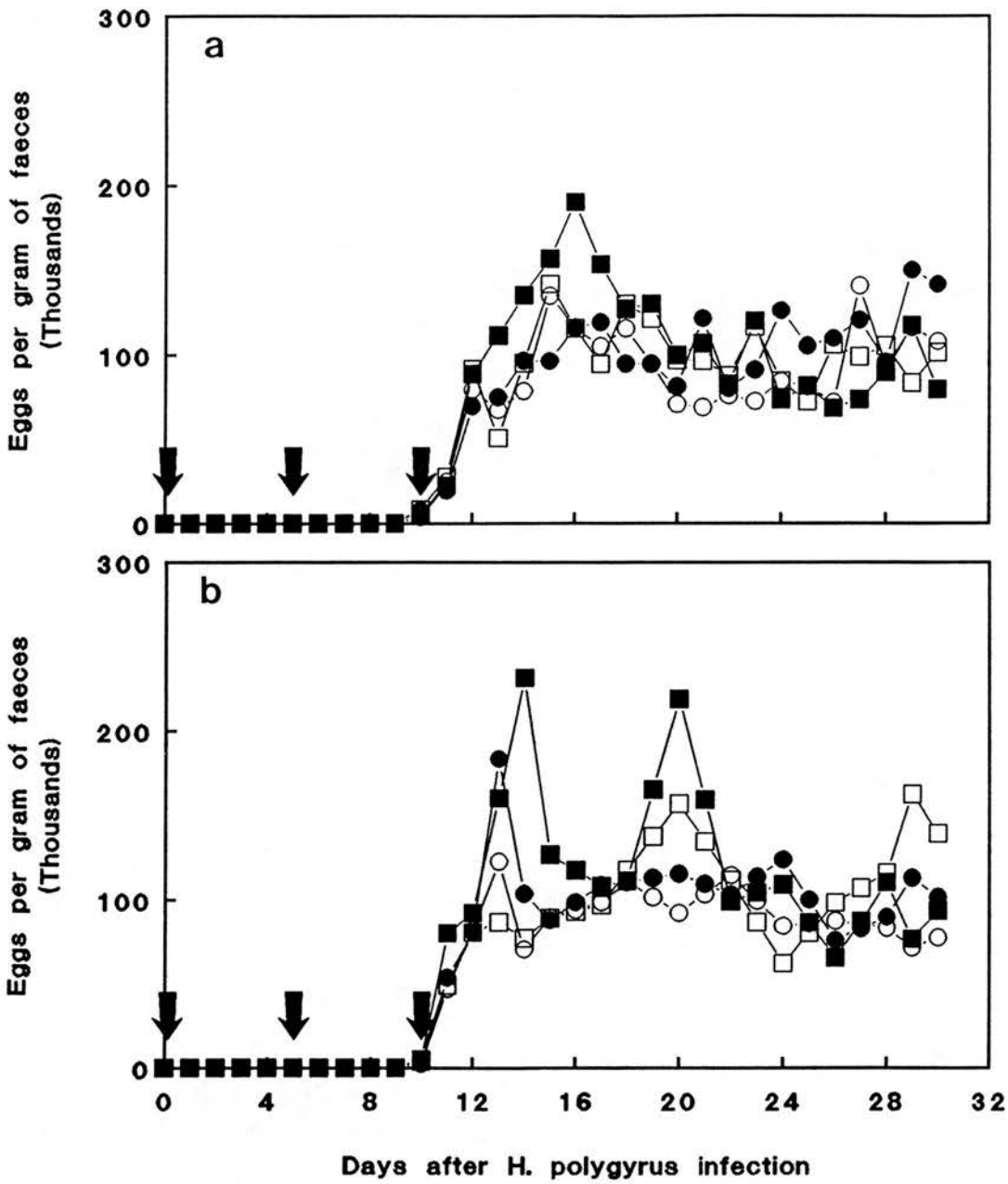

FIGURE 8.1 The faecal worm egg count of mice infected *H. polygyrus* alone (●) and those conjointly infected with *T. congolense* on day 0 (○), day 5 (■) and day 10 (□) after *H. polygyrus* infection in Experiment 8.1 (a) and Experiment 8.2 (b). Arrows indicate times of infection with *T. congolense*

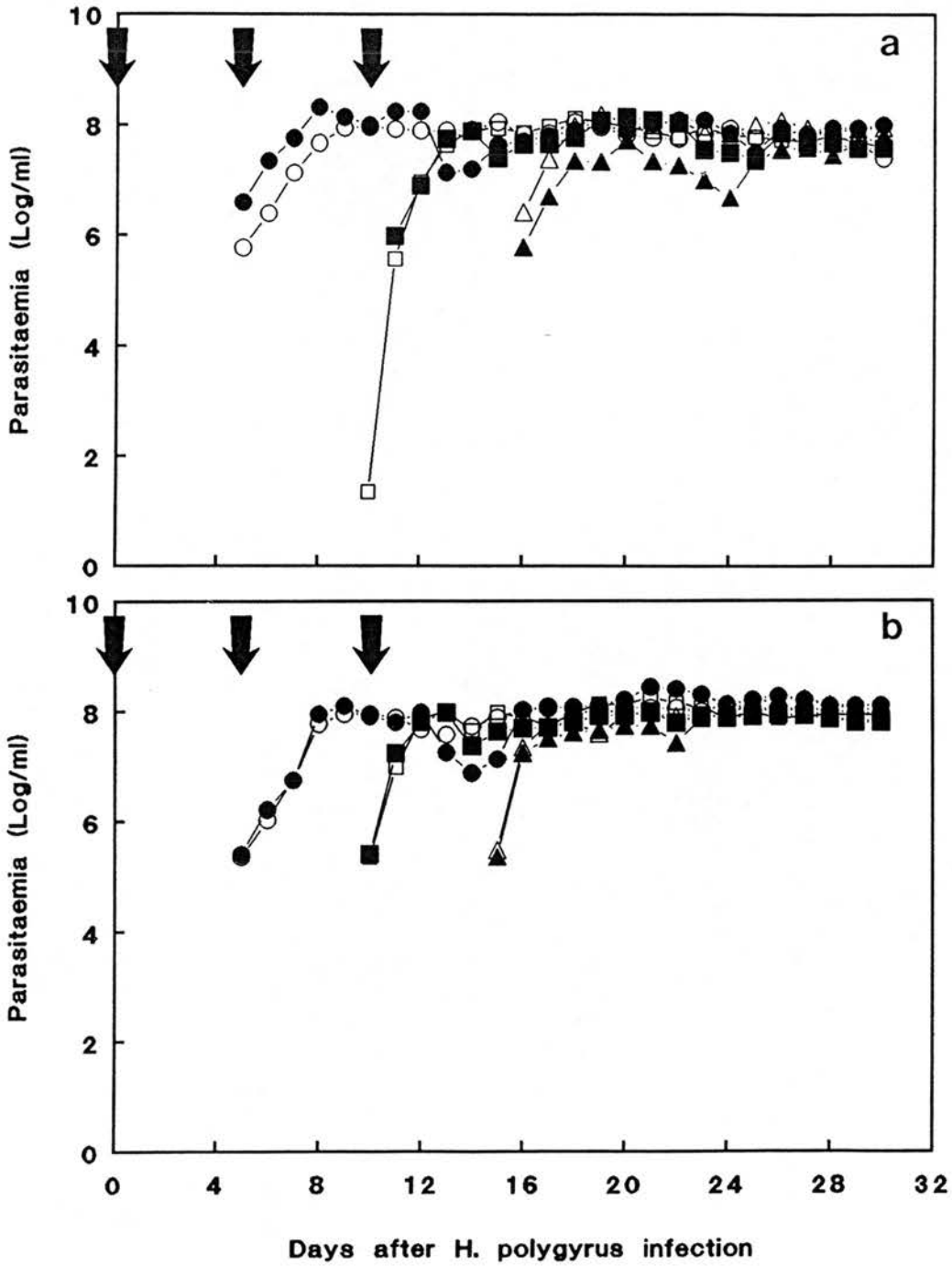

FIGURE 8.2 The mean parasitaemia of surviving mice conjointly infected with *T. congolense* on day 0 (●), day 5 (■) and day 10 (▲) after *H. polygyrus* and their respective controls infected with *T. congolense* alone on day 0 (○), day 5 (□) and day 10 (△) of Experiments 8.1 (a) and 8.2 (b). Arrows indicate the times of infection with *T. congolense*

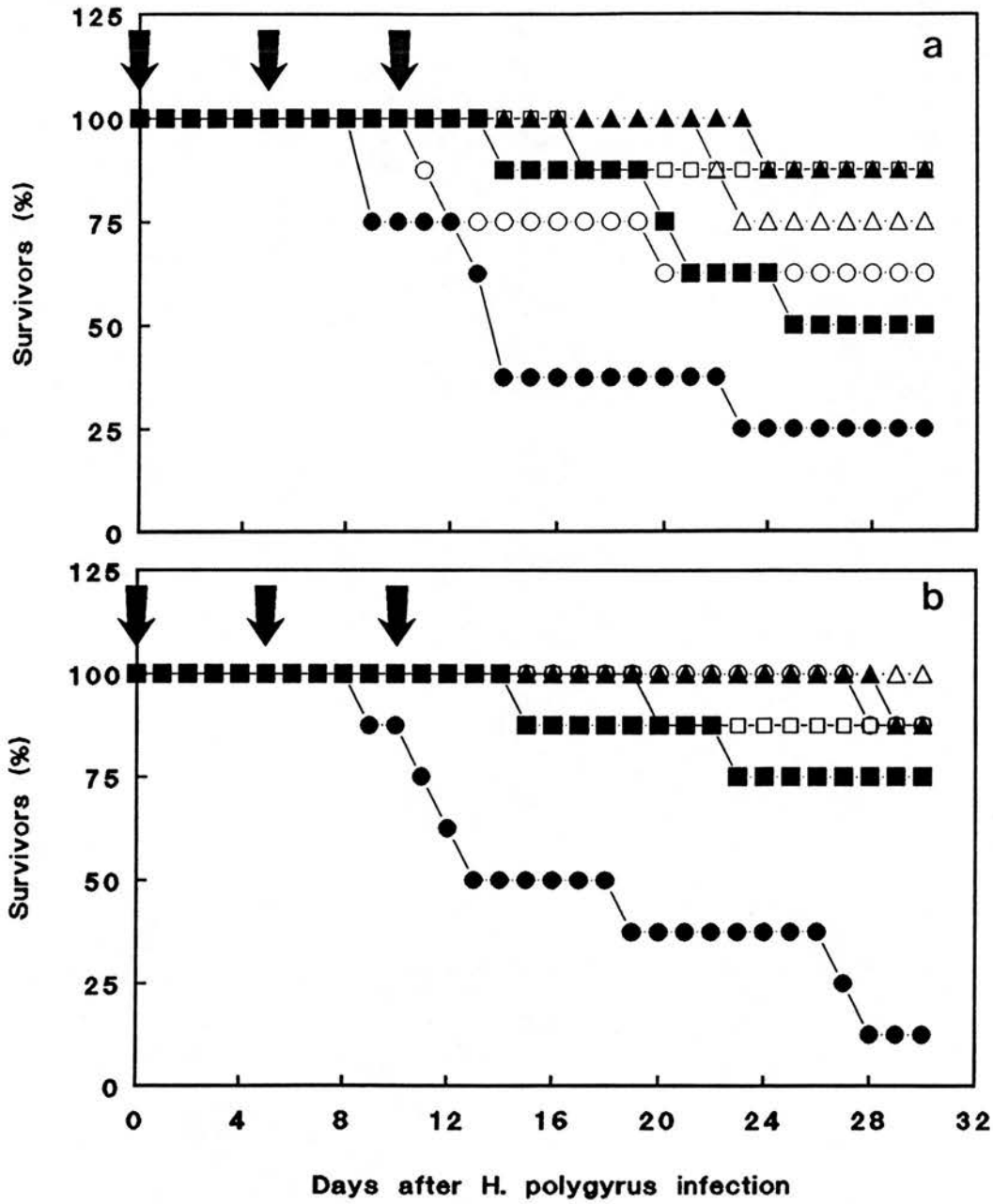

FIGURE 8.3 The mortality of mice conjointly infected with *T. congolense* on day 0 (●), day 5 (■) and day 10 (▲) after *H. polygyrus* and their respective controls infected with *T. congolense* alone on day 0 (○), day 5 (□) and day 10 (△) of Experiments 8.1 (a) and 8.2 (b). Arrows indicate the times of infection with *T. congolense*

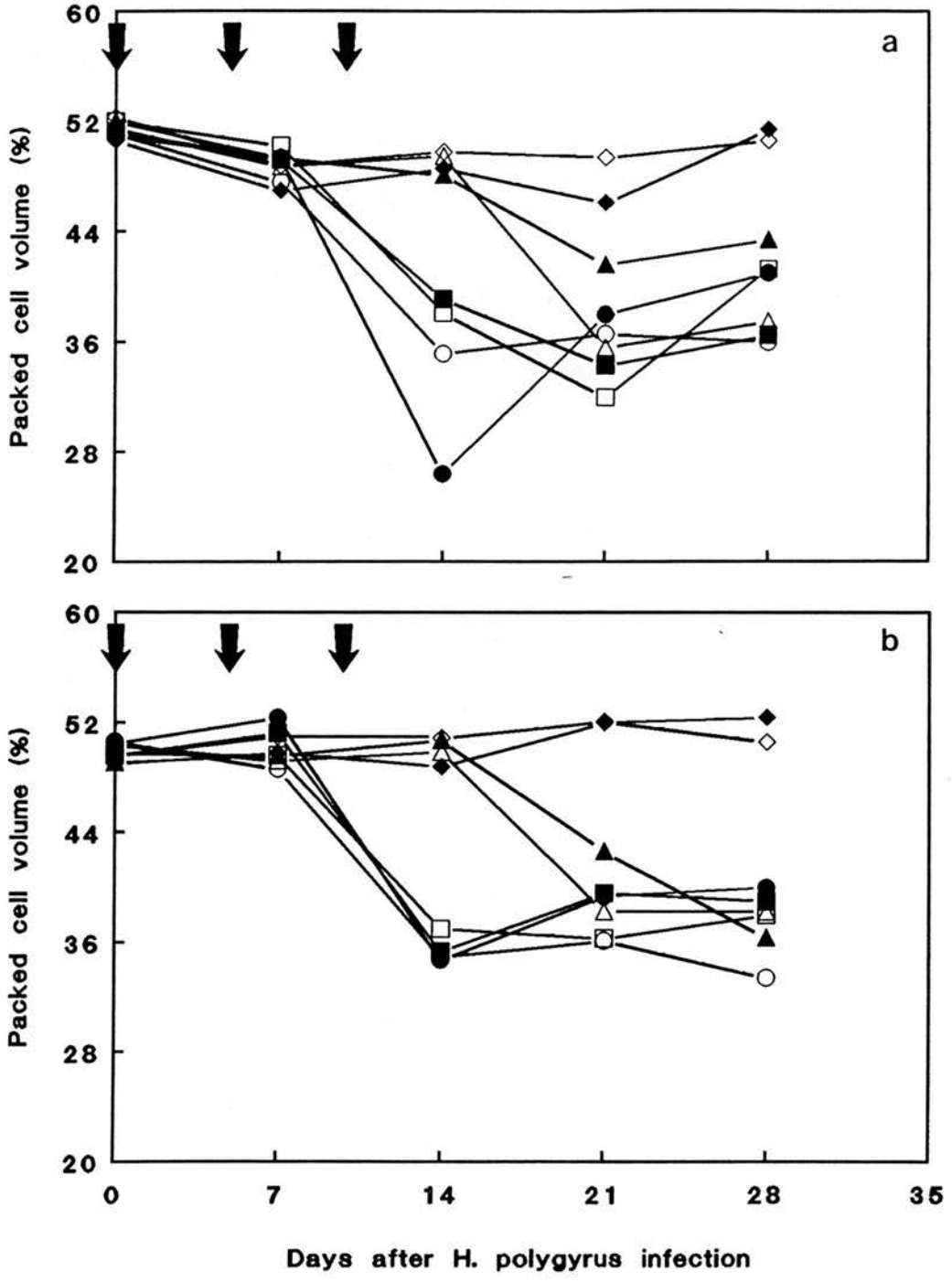

FIGURE 8.4 The mean PCV of mice not infected (◇) or infected with *H. polygyrus* alone (◆) or conjointly infected with *T. congolense* on day 0 (●), day 5 (■) and day 10 (▲) after *H. polygyrus* infection and their respective controls infected with *T. congolense* alone on day 0 (○), day 5 (□) and day 10 (△) of Experiments 8.1 (a) and 8.2 (b). Arrows indicate the times of infection with *T. congolense*

There was very little variation in the PCVs of mice infected with only *H. polygyrus* or uninfected throughout the experiment (Figures 8.4a,b). Mice with dual infection or only the trypanosome infection were anaemic from about two weeks after infection with *T. congolense*. In Experiment 8.1 the decrease in PCV at 14 DAI was not significantly greater in animals dually infected at 0 DAI as compared to the similar group in Experiment 8.2 (U=30, P=0.1111).

All groups of experimental animals showed an increase in live weight, with little or no variation between the groups during the course of the experiment (Figure 8.5a,b). The weights of the spleens from mice with dual infections and their respective trypanosome-infected controls were similar and significantly higher (P<0.05) than the uninfected controls. There was no significant difference (U=68, P>0.05) in the weights of the spleens of mice infected with *H. polygyrus* alone and the uninfected controls (Figures 8.6a,b). Generally, the spleen weights increased in proportion to the duration of *T. congolense* infection.

Worm establishment in the mice was variable. All the worms recovered were adults and predominantly females (Table 8.2). The lengths of male worms from the various groups were similar. Female worms from mice that were conjointly infected with both parasites at day 10 were significantly shorter than those infected with only *H. polygyrus* infection in Experiments 8.1 and 8.2 (Table 8.3) ($F_{1,28}$=84.05. P<0.0001; $F_{1,38}$=4.49, P=0.031 respectively) The *in vitro* egg production by female worms from the different groups were somewhat variable but with similar ranges in both experiments (Table 8.4).

TABLE 8.2 The mean worm burdens (+SEM) and the male/female (M:F) ratios of worms in TO mice infected conjointly with *H. polygyrus* and *T. congolense* or *H. polygyrus* alone in Experiments 8.1 and 8.2

Group	Mean (SEM)worm burden of survivors							
	Experiment 8.1				Experiment 8.2			
	No.	Total		M:F	No.	Total		M:F
Conjoint (day-0)	2	336.0	(8)	0.69	1	296.0	(0)	0.74
Conjoint (day-5)	4	282.3	(19)	0.53	6	348.3	(8)	0.85
Conjoint (day-10)	7	341.6	(26)	0.60	7	331.4	(23)	1.00
Nematode alone	8	292.0	(8)	0.73	8	346.4	(20)	0.92

TABLE 8.3 The mean lengths (+SEM) of male and females worms from mice with primary *H. polygyrus* infection and those with concomitant *T. congolense* infection during different stages of *H. polygyrus* infection in Experiments 8.1 and 8.2.

Group	Mean (SEM) length of worm in millimetres							
	Experiment 8.1				Experiment 8.2			
	Male		Female		Male		Female	
Conjoint (day-0)	6.6	(0.1)	16.9	(0.3)	7.1	(0.1)	16.9	(0.4)
Conjoint (day-5)	7.3	(0.1)	16.4	(0.2)	7.0	(0.1)	16.6	(0.3)
Conjoint (day-10)	6.7	(0.1)	13.9	(0.4)	6.6	(0.1)	14.4	(0.2)
Nematode alone	7.0	(0.2)	18.0	(0.2)	6.5	(0.1)	15.4	(0.4)

TABLE 8.4 The mean 24 hour *in vitro* egg production of female *H. polygyrus* from control mice and those with conjoint *H. polygyrus* and *T. congolense* infections in Experiments 8.1 and 8.2

Group	Mean number of eggs/worm/24 h (SEM)			
	Experiment 8.1		Experiment 8.2	
Conjoint (day-0)	274.5	(24)	282.4	(0)
Conjoint (day-5)	253.5	(32)	235.3	(22)
Conjoint (day-10)	319.4	(47)	248.8	(38)
Nematode alone	250.0	(33)	293.6	(27)

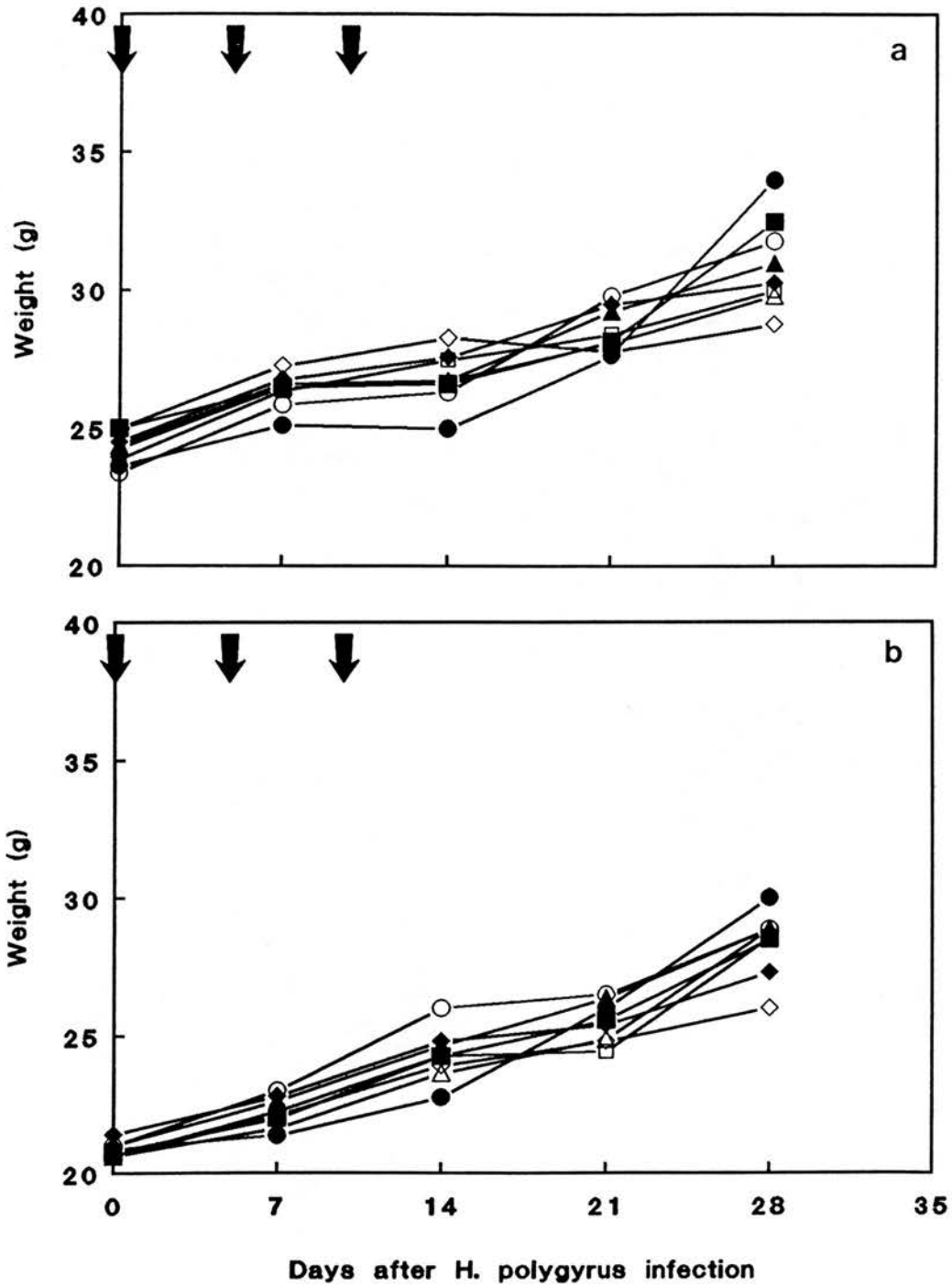

FIGURE 8.5 The mean weights of mice not infected (◇) or infected with *H. polygyrus* alone (◇) or conjointly infected with *T. congolense* on day 0 (●), day 5 (■) and day 10 (▲) after *H. polygyrus* infection and their respective controls infected with *T. congolense* alone on day 0 (○), day 5 (□) and day 10 (△) of Experiments 8.1 (a) and 8.2 (b). Arrows indicate the times of infection with *T. congolense*

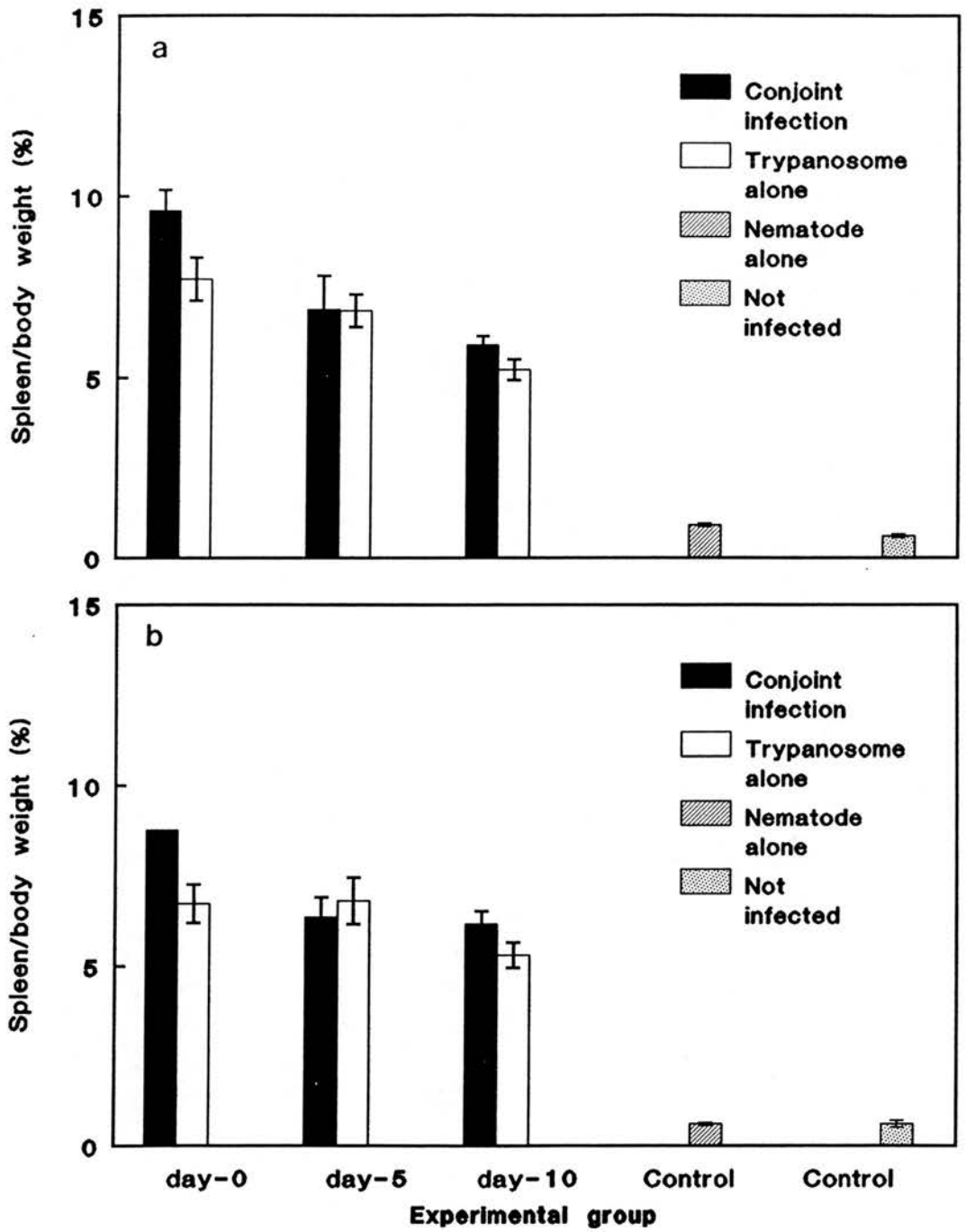

FIGURE 8.6 The mean (±SEM) spleen weights as a proportion of body weights of uninfected mice and those conjointly infected with *H. polygyrus* and *T. congolense* and their respective *T. congolense*-infected controls in Experiments 8.1 (a) and 8.2 (b).

For logistic reasons ELISA studies were only done in Experiment 8.1. The antibody response to worm infection was evident from 14 DAI. *T. congolense* infected mice and uninfected controls did not show any anti-*H. polygyrus* response. The anti-*H. polygyrus* responses were generally depressed in mice having conjoint infections, particularly so as the infection progressed.

A gradually increasing antibody response to *T. congolense* infection was recorded in mice from about day 7 after *T. congolense* infection (Figures 8.8a,b,c). Those infected with only the nematode and the uninfected control were unresponsive to *T. congolense*-derived antigens. The responses obtained from mice conjointly infected on day 0 and day 5 were consistently lower significantly than those of their respective trypanosome-infected controls (e.g. U=3, P=0.0212 for the day-5 group). There was no apparent difference between those conjointly infected on day 10 and their *T. congolense*-infected controls (U=16, P>0.05).

8.4 DISCUSSION

The results obtained in this study have shown that mice carrying a larval *H. polygyrus* infection were severely compromised when they were superinfected with *T. congolense*. This was shown by the enhanced mortality and reduced antibody response against both *H. polygyrus* and *T. congolense* antigens. This suggests a synergistic interaction between the two parasites. However, mice with an adult infection of *H. polygyrus* were not so compromised when they were conjointly infected with *T. congolense*, instead the female worms from these mice were comparatively shorter than those of the other groups.

The clinicopathological outcome of the interaction seemed to have been determined mainly by the *T. congolense*. The anaemia was primarily due to *T. congolense* infection, as the PCV in mice infected with *T. congolense* alone was not higher than in those with conjoint infection. Moreover, mice infected with *H. polygyrus* alone had similar PCV's to the uninfected controls.

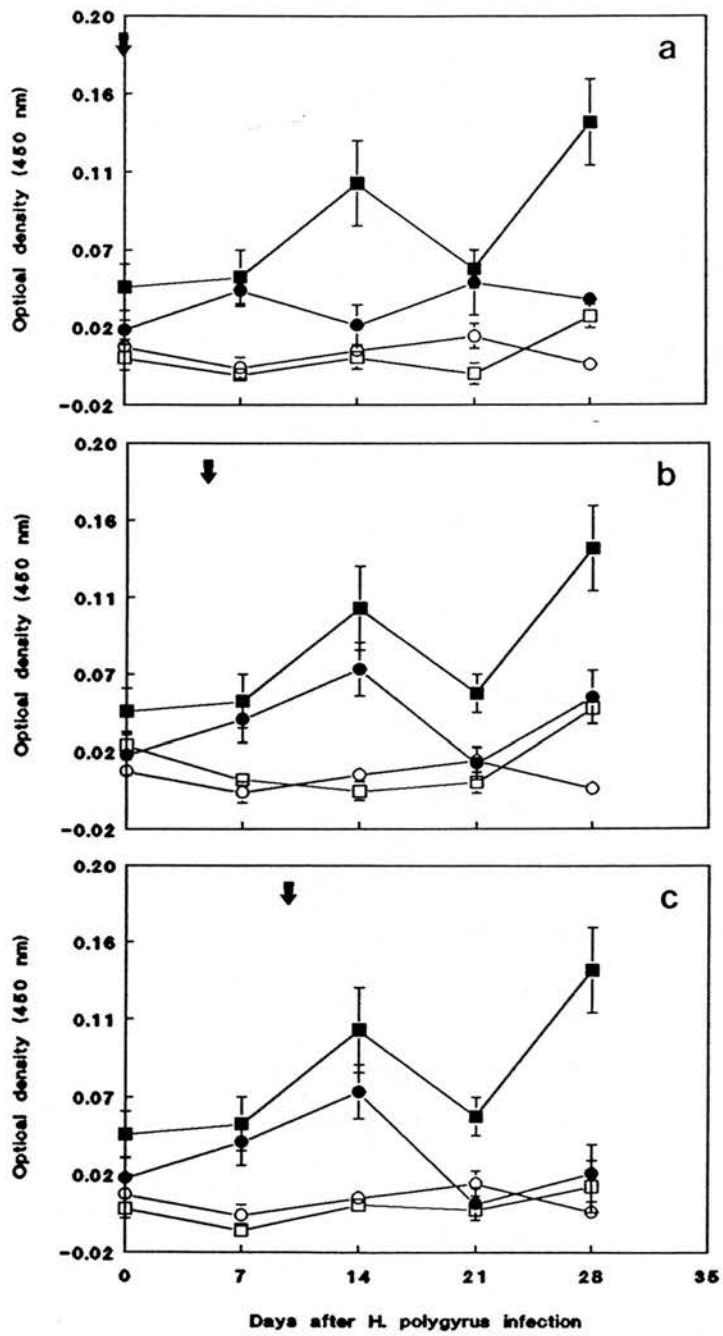

FIGURE 8.7 The antibody responses against *H. polygyrus*-derived antigens by mice infected with *T. congolense* on day 0 (a), day 5 (b) and day 10 (c) after *H. polygyrus* infection in Experiment 8.1. ● = conjoint infection, □ = *T. congolense* alone, ■ = *H. polygyrus* alone, ○ = uninfected control. Arrows indicate the times of infection with *T. congolense*

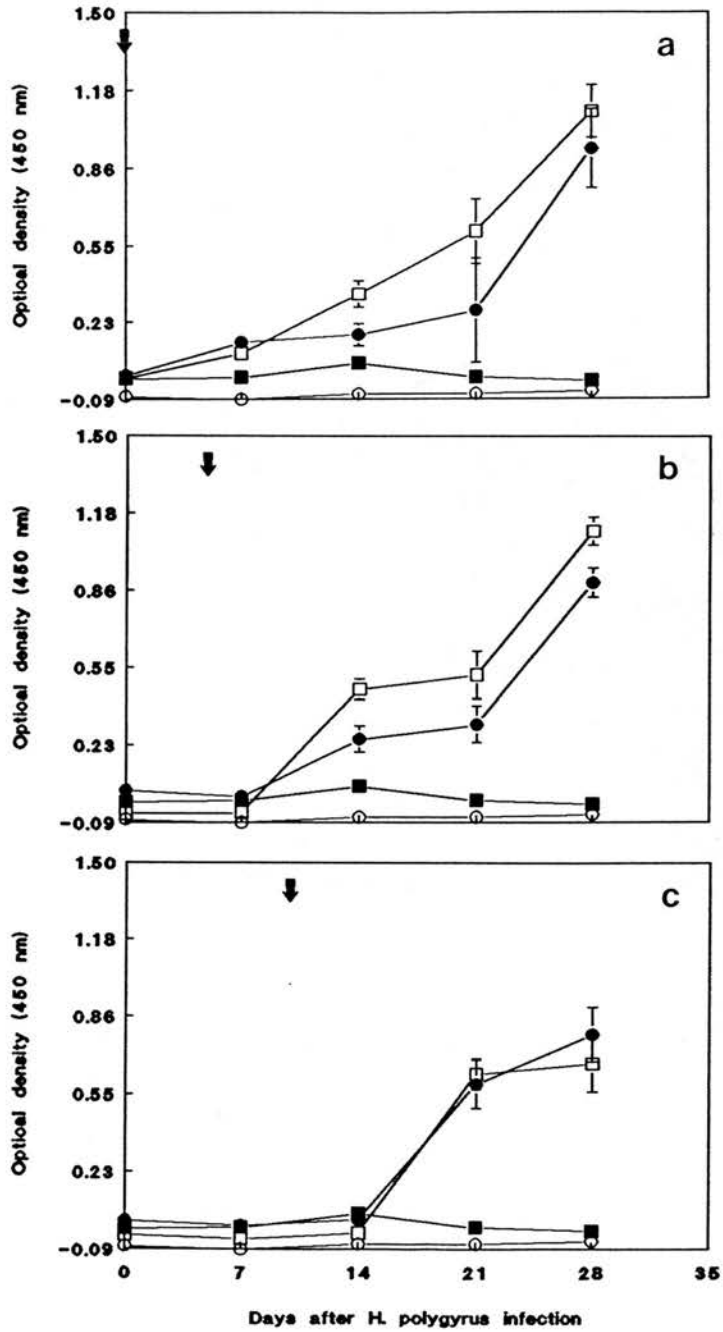

FIGURE 8.8 The antibody responses against *T. congolense*-derived antigens by mice infected with *T. congolense* on day 0 (a), day 5 (b) and day 10 (c) after *H. polygyrus* infection in Experiment 8.1. ●= conjoint infection, □ = *T. congolense* alone, ■= *H. polygyrus* alone, ○= uninfected control. Arrows indicate the times of infection with *T. congolense*

The pathology associated with the larval development and the disruptive emergence of juvenile worms may be responsible for the enhanced mortality of mice infected with *T. congolense* 0 or 5 days after *H. polygyrus* infection.

Although the live weights of *T. congolense*- and *H. polygyrus*-infected mice did not differ, the weights of the spleens (see Figure 8.6a,b), suggest that the eviscerated carcass weight of the mice infected with *T. congolense* may be greatly reduced since a large proportion of their weight could be accounted for by the enlargement of the lymphoid organs, as shown in Chapter 10.

Trypanosoma congolense parasitaemias in mice are similar irrespective of the treatment whereas the effects of a concomitant infection can easily be distinguished with other protozoa such as *T. musculi* and *B. microti* which have defined phases of parasitaemia (Bell *et al.*, 1984a,b; Nichol and Sewell, 1984). Thus effects of *H. polygyrus* on the course of *T. congolense* may not be apparent from the daily parasitaemia as recorded in the present study. Studies have shown that *T. spiralis* and *H. polygyrus* enhanced resistance to *P. berghei* and *B. microti* respectively (Ngwenya, 1982; Mzembe, *et al.*, 1984). These authors suggested that this was due to a macrophage-mediated, non-specific immunity to the protozoan induced by helminth infection and a similar reason may be responsible for the slightly low parasitaemia observed in mice carrying 10 day old *H. polygyrus* infection when they were superinfected with *T. congolense*, especially in Experiment 8.1.

In the present experiment, there was no consistent difference between the worm burdens in conjointly infected mice compared with the controls, suggesting that *T. congolense* did not influence the establishment of *H. polygyrus*. Presumably, since *T. congolense* was never given before *H. polygyrus*, the immunosuppressive effect of *T. congolense* was not in place at the crucial phase of the helminth establishment.

It might have been expected that, since *T. congolense* is known to cause nonspecific immunosuppression (Bancroft and Askonas, 1985; Morrison, Murray and Akol, 1985), *H. polygyrus* would have grown larger in conjointly infected mice. On the contrary, the male worms were unaffected but the female worms tended to be shorter, especially in mice with 10 day old infection at superinfection with *T. congolense*. The stunted growth of worms might have been due to an enhanced macrophage-mediated non-specific resistance as suggested for the reduced growth of *Hymenolepis diminuta* in rats concurrently infected with *T. lewisi* or *P. berghei* (Rigby and Chobotar, 1966; Fenwick, 1980). Stunting of female *H. polygyrus*, however, did not appear to influence the fecundity of the worms as seen from the number of eggs passed *in vitro* (Table 8.3).

In addition to interfering with the host's response at the intestinal level against homologous antigens (Behnke *et al.*, 1983; Pritchard, Ali and Behnke, 1984; Dobson *et al.*, 1985; Pritchard and Behnke, 1985) and delaying the expulsion of other nematodes in concurrent infections (Jenkins and Behnke, 1977; Behnke, Wakelin and Wilson, 1978; Hagan and Wakelin, 1982), *H. polygyrus* is also known to depress immunity to unrelated antigens such as sheep red blood cells (SRBC) and viruses (Chowaniec *et al.*, 1972; Shimp *et al.*, 1975; Ali and Behnke, 1983). The consistently lower antibody titre to *T. congolense* antigens therefore agrees with earlier studies. The results also provide further evidence that infection with *T. congolense* induces a significant immunosuppression of the host. Irrespective of the time of conjoint infection with *T. congolense* in *H. polygyrus* infected mice, there was a marked depression in the antibody response to *H. polygyrus*. In *T. brucei* infection in mice, such an impaired response has been attributed to suppressor macrophages and T-cells which arise during infection (Murray, Jennings, Murray and Urquhart, 1974b,c; Borowy, Sternberg, Schreiber, Nonnengasser and Overath, 1990) and to possible depletion of T- and B-memory cells (Askonas, Corsini, Clayton and Ogilvie, 1979).

In conclusion, concurrent infection with *T. congolense* during the larval phase of a *H. polygyrus* infection resulted in a synergistic interaction which was more detrimental to TO mice than a similar protozoal infection during the adult phase of infection with the helminth.

CHAPTER NINE

THE EFFECT OF CONJOINT *HELIGMOSOMOIDES POLYGYRUS* AND *TRYPANOSOMA CONGOLENSE* INFECTION IN 'TO' MICE IMMUNIZED AGAINST SECONDARY *H. POLYGYRUS* INFECTION BY AN ABBREVIATED ADULT INFECTION

9.1 INTRODUCTION

Sporadic outbreaks of clinical helminthosis are not uncommon in the tropics and these have been attributed to various factors which result in breakdown of host resistance (Chiejina, 1986). One such factor may be immunodepression induced by various species of trypanosomes during an intercurrent infection (Fakae and Chiejina, 1993). Since the experiments detailed in Chapter 7 indicated that resistance to homologous *H. polygyrus* challenge was correlated with antibody titre as well as with some peripheral leucocytic responses, the experiments in this and the following chapter examined these and other clinicopathological parameters as well as the growth parameters of the nematode and *T. congolense* during concurrent infections in mice previously immunized against *H. polygyrus* by termination of an adult infection.

9.2 MATERIALS AND METHODS

Sixty four female TO mice were randomly selected into eight groups of eight mice each. They were either unimmunized or immunized by termination of an adult infection (see Section 7.2.1) and were infected concurrently with standard doses of 500 L_3 of *H. polygyrus* and 10^4 blood stream forms of *T. congolense* orally and intraperitoneally respective or with each parasite alone or not infected as detailed in Table 9.1.

Mice were monitored daily for faecal worm egg counts and *T. congolense* parasitaemias as applicable. Following the start of the challenge infection, weekly PCVs, differential leucocyte counts on Giemsa stained blood smears and live weights were determined for each mouse. Individual serum samples were collected weekly for ELISA.

The survival of the mice was recorded daily until the experiment was terminated 30 days after challenge infection. The total worm burdens (males and females) and spleen weights were determined post mortem. The length of the surviving worms were determined from pooled-group samples.

TABLE 9.1 Experimental plan for immunization of TO mice by abbreviation of adult *H. polygyrus* infection and subsequent challenge with *H. polygyrus* and *T. congolense*

Group	Primary infection (Day -20)	Anthelmintic treatment (Day -8)	Challenge infection (Day 0)	
			Nematode	Trypanosome
Immunized/Conjoint	+	+	+	+
Immunized/Nematode	+	+	+	-
Immunized/Trypanosome	+	+	-	+
Immunized/Control	+	+	-	-
Unimmunized/Conjoint	-	+	+	+
Unimmunized/Nematode	-	+	+	-
Unimmunized/Trypanosome	-	+	-	+
Unimmunized/Control	-	+	-	-

9.3 RESULTS

The worm egg counts from immunized animals were markedly fewer than those from the unimmunized. The unimmunized mice infected with both *H. polygyrus* and *T. congolense* or *H. polygyrus* alone showed similar EPG. In the immunized mice, however, the egg counts in conjointly infected mice were generally higher than those infected with *H. polygyrus* alone (Figure 9.1).

All the groups infected with *T. congolense* were parasitaemic from 5 DAI and rapidly rose to attain a plateau between 8 to 10 DAI. The parasitaemias in the various groups were almost indistinguishable (Figure 9.2a). All the mice which were infected with *H. polygyrus* alone and all the uninfected controls, survived till the end of the experiments. The onset of death was earlier and the overall mortality was more in unimmunized than in immunized mice and immunized mice with conjoint infection had earlier death than their controls infected with *T. congolense* alone. However, the unimmunized mice infected with *T. congolense* alone

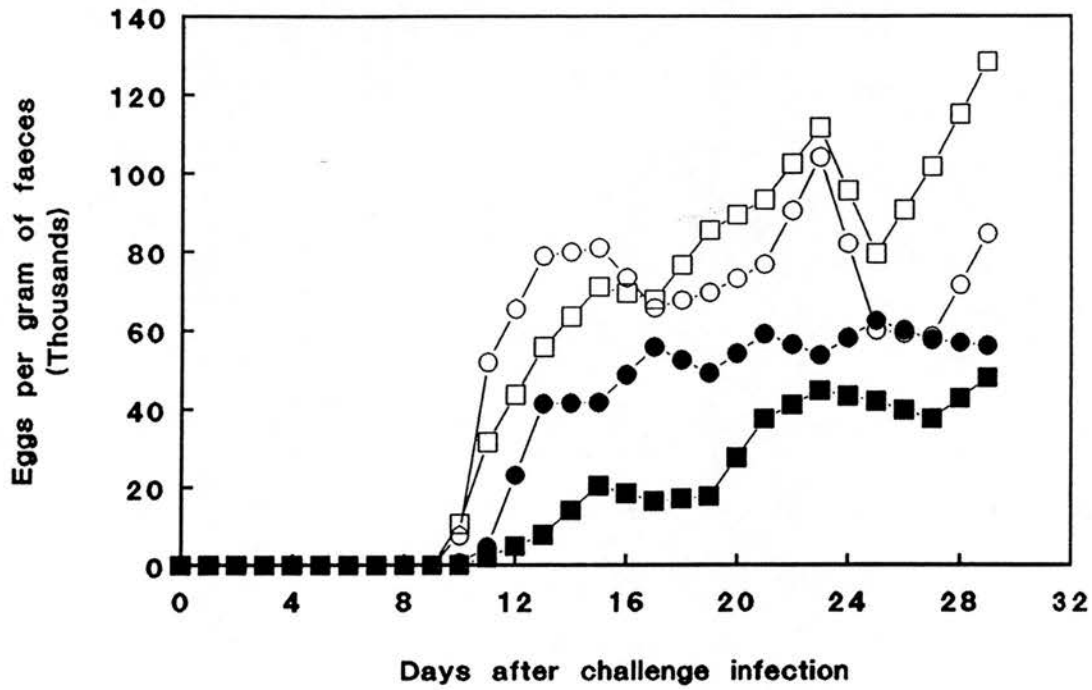

FIGURE 9.1 Faecal egg counts in immunized (● , ■) and unimmunized (○ , □) mice challenge with *H. polygyrus* alone (■ , □) or together with *T. congolense* (●, ○)

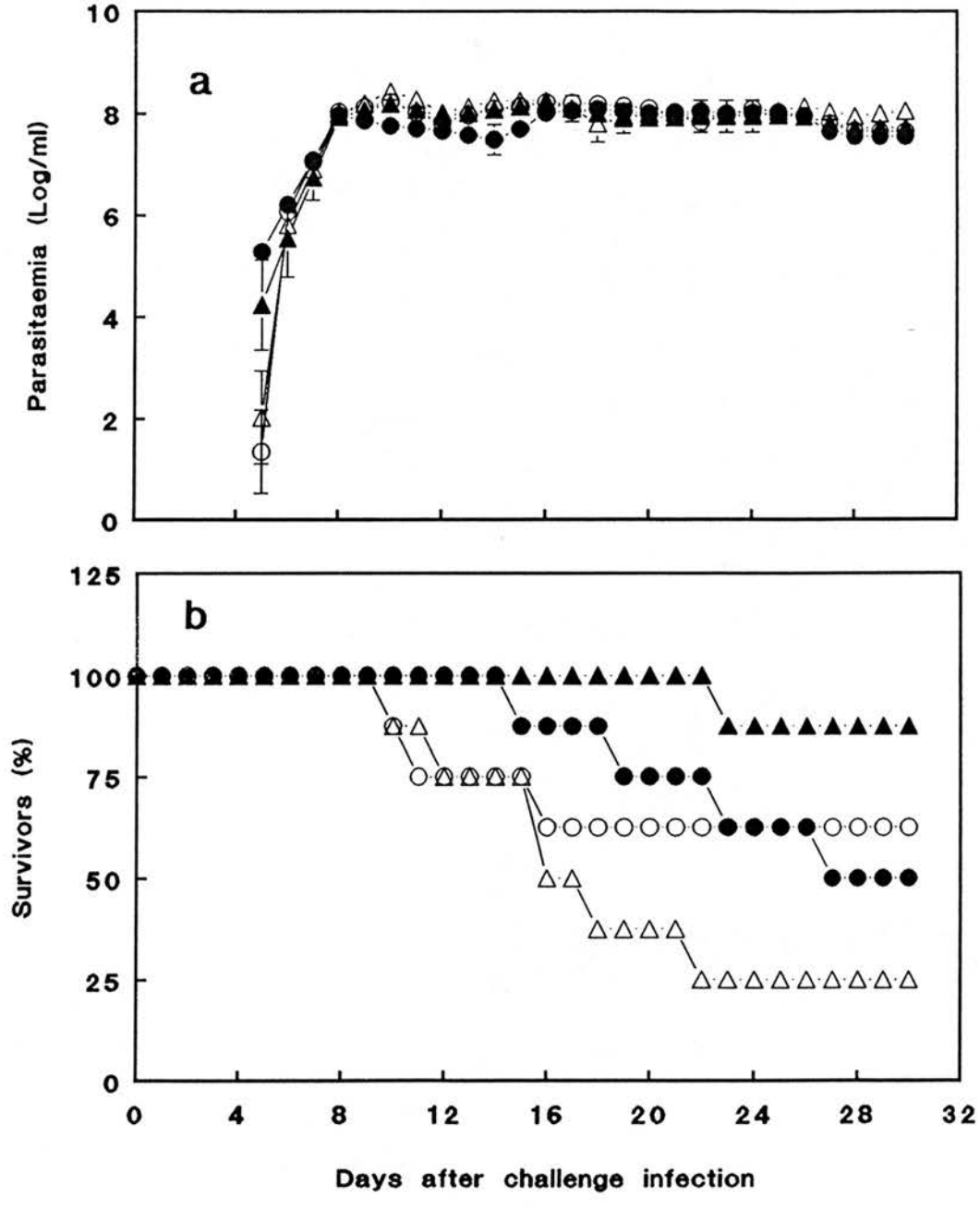

FIGURE 9.2 Mean (±SEM) *T. congolense* parasitaemia (a) and survival (b) of immunized (● , ▲) or unimmunized (○ , △), challenged with *T. congolense* alone (▲, △) or together with *H. polygyrus* (●, ○)

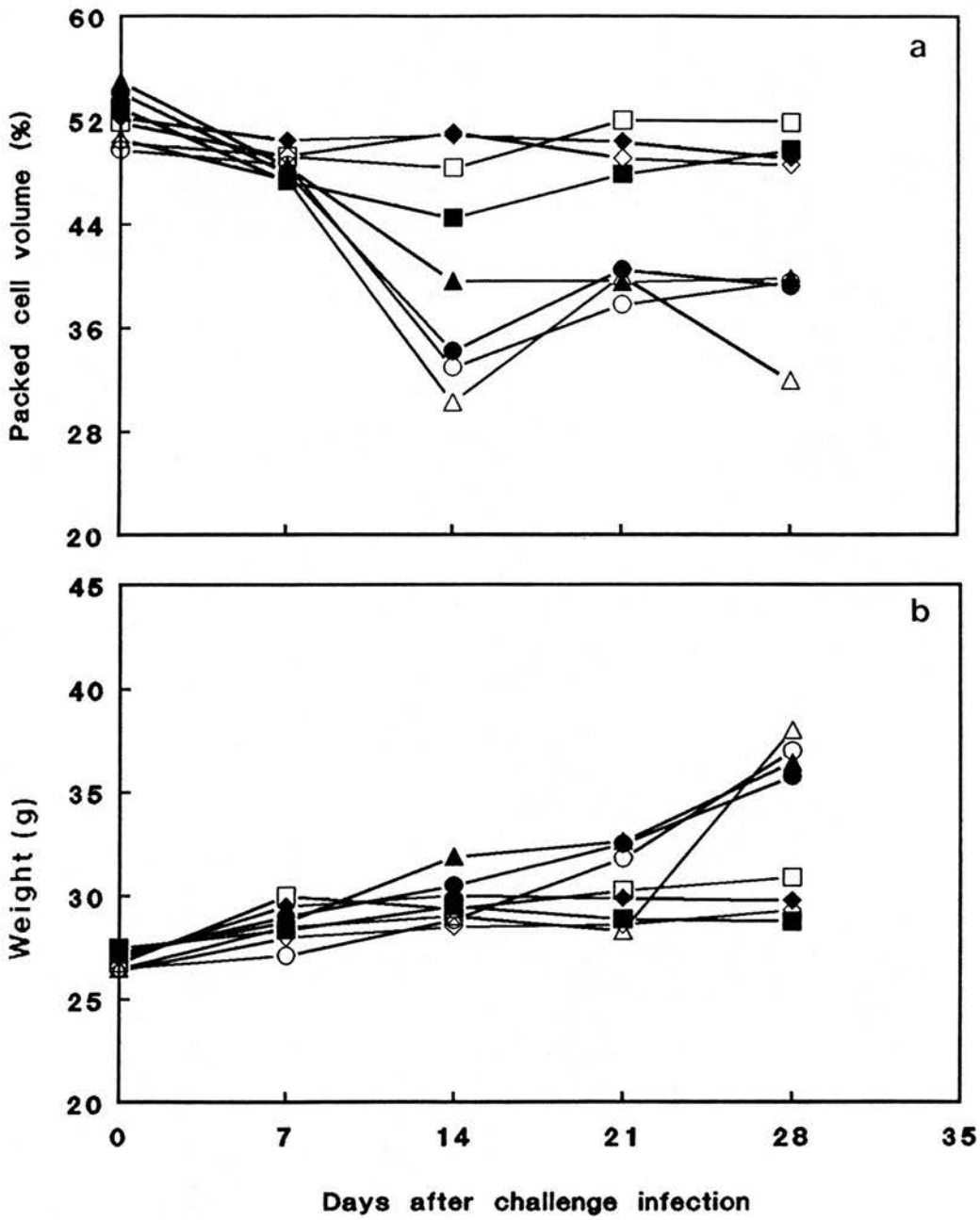

FIGURE 9.3 The mean packed cell volume (a) and mean live weights (b) of immunized (●, ■, ▲, ◆) or unimmunized (○, □, △, ◇) mice, uninfected (◆, ◇) or challenged with *T. congolense* alone (▲, △) or *H. polygyrus* alone (■, □) or together with *T. congolense* (●, ○)

underwent even greater mortality with only 25% of the mice surviving at the end of the experiment (Figure 9.2b).

All mice infected with *T. congolense* irrespective of the immune status, unlike all the uninfected and those infected with *H. polygyrus* alone, became anaemic from 14 DAI till the end of the experiment (Figure 9.3a), with the difference between their PCVs and their respective controls being significantly different ($P<0.05$).

There were little or no apparent change in the live weight of all uninfected mice and those infected with only *H. polygyrus* but there was a general gradual increase in the weight of mice which were conjointly infected or infected with only *T. congolense* (Figure 9.3b). The spleen contributed far more to the carcass weight in mice infected with *T. congolense* than in those infected with *H. polygyrus* alone or uninfected, these irrespective of the immune status of the animals (Figure 9.4). The spleens of the unimmunized animals were consistently larger than their respective controls and were statistically significant ($P<0.05$)

The unimmunized control infected with *H. polygyrus* alone harboured 263 adult worms (120 males and 143 females). While the mice conjointly infected, immunized and unimmunized, had mean worm burdens of 254 and 270 respectively, immunized mice challenged with only *H. polygyrus* had only 106 worms. Thus there was abrogation of immunity to *H. polygyrus* during conjoint infection with *T. congolense* (Figure 9.5).

Worms from immunized mice, either conjointly infected or infected with *H. polygyrus* alone produced similar number of eggs *in vitro* which were generally lower than their respective controls from unimmunized mice, and significantly so in those infected with *H. polygyrus* alone ($U=11$, $P=0.0141$) (Figure 9.6).

The lengths of the males were similar in all experimental groups (Figure 9.7a). Female worms from unimmunized mice tended to be larger than those from

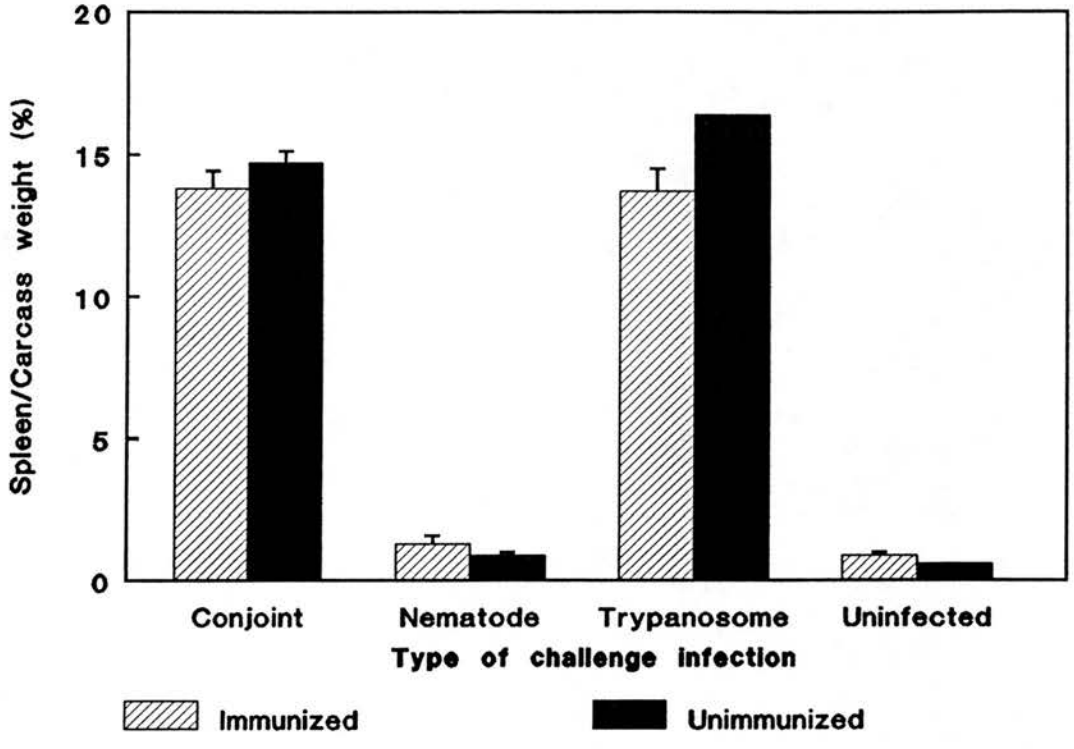

FIGURE 9.4 Mean (±SEM) weights of spleen as percentage of carcass weights of immunized or unimmunized mice, uninfected or infected with *T. congolense* alone or with *H. polygyrus* alone or together with *T. congolense*

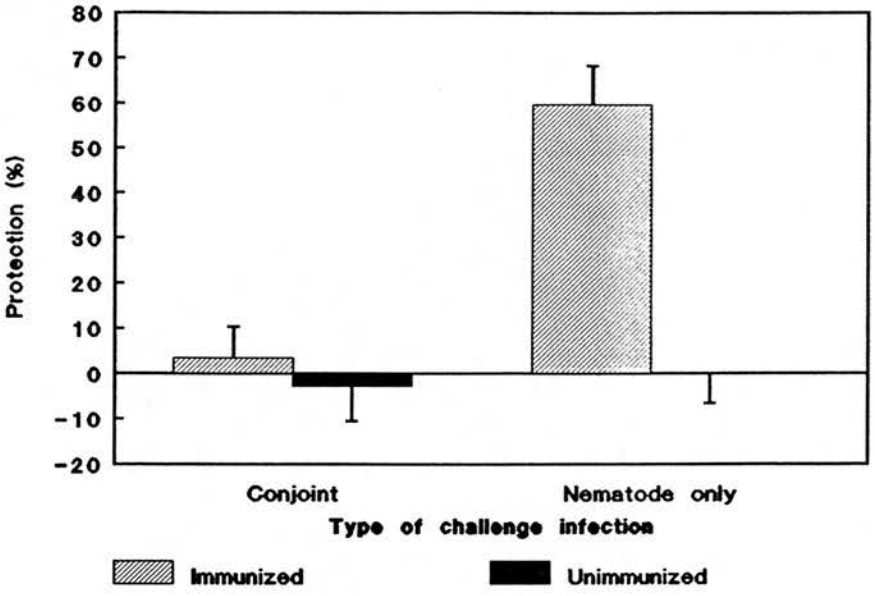

FIGURE 9.5 Mean (±SEM) protection against homologous *H. polygyrus* challenge in immunized or unimmunized mice infected with *H. polygyrus* alone or together with *H. polygyrus*

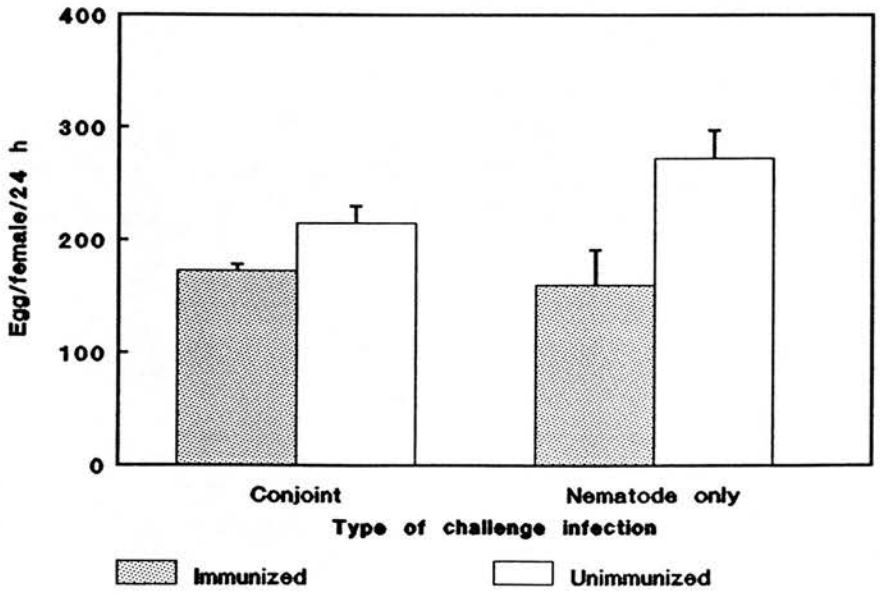

FIGURE 9.6 Mean (±SEM) number of eggs passed in vitro by female *H. polygyrus* from immunized or unimmunized mice infected with *H. polygyrus* alone or together with *T. congolense*

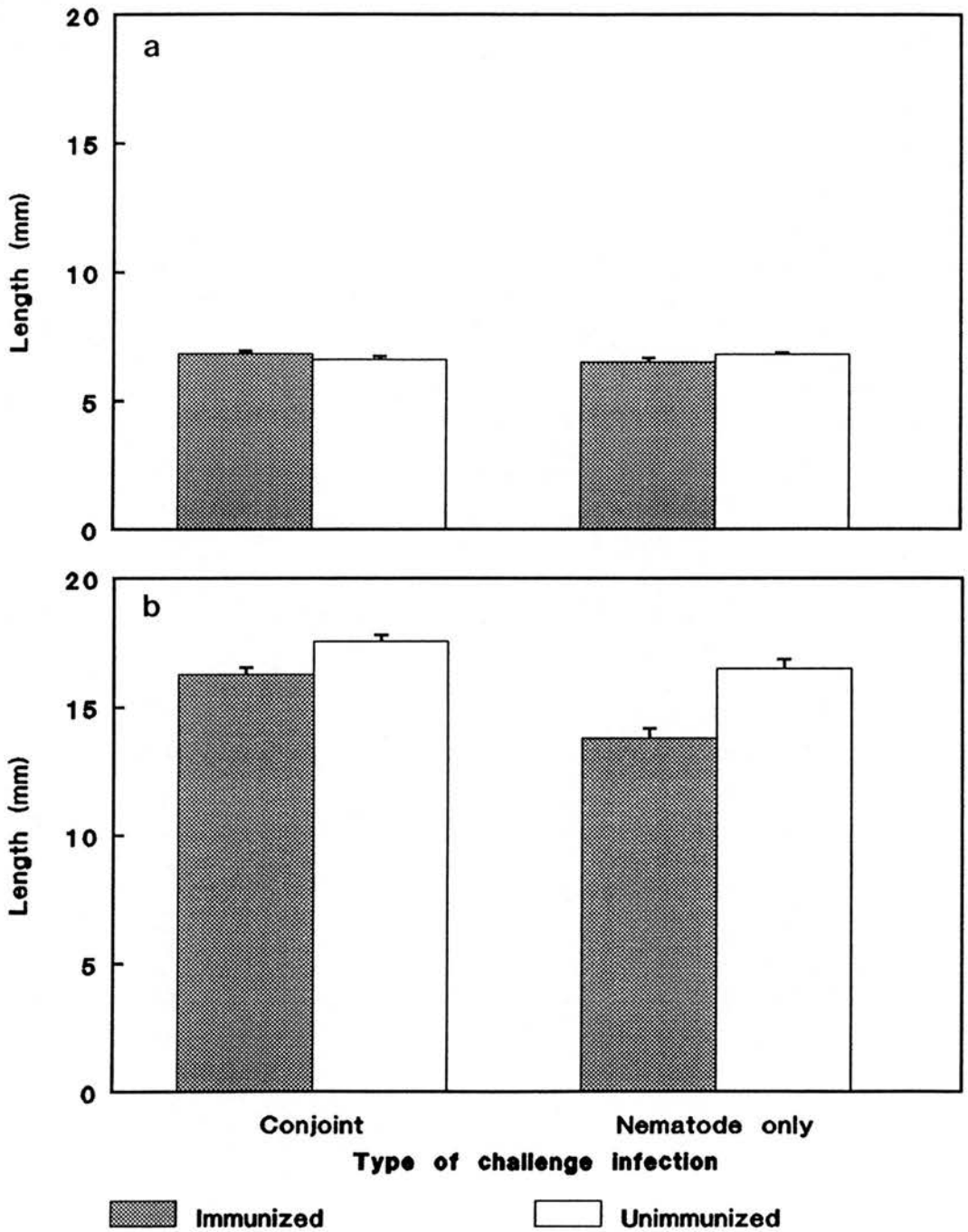

FIGURE 9.7 Mean (±SEM) lengths of male (a) and female (b) worms from immunized or unimmunized mice infected with *H. polygyrus* alone or together with *T. congolense*

immunized mice and those from conjointly infected mice were significantly larger than their controls from similar mice infected with only *H. polygyrus* (Figure 9.7b) ($F_{1,38}$=26.896, P<0.0001; $F_{1,38}$=6.074, P=0.018 for immunized and unimminuzed groups respectively).

Mice experiencing primary infection with *H. polygyrus* had a transient eosinophilia but the rise in the proportion of peripheral eosinophils in immunized mice was sustained. No apparent change was observed in mice infected with *T. congolense* alone (Figures 9.8a). By 28 DAI, the eosinophilia in immunized mice with conjoint infection was significantly lower than its control infected with *H. polygyrus* alone (U=0.0, P=0.002).

There was a general decline in the proportion of circulating lymphocytes with progressing infection but the decline which were similar in animals with conjoint and single trypanosome infections, were more drastic although not significantly so, than in their respective controls infected with *H. polygyrus* alone (P>0.05). The changes were not affected by the immune status of mice to *H. polygyrus* (Figure 9.8b).

No apparent change occurred in the peripheral monocytosis in uninfected mice and those infected with only *H. polygyrus* but a steady rise was observed in mice infected with *T. congolense* alone or conjointly (Figure 9.9a). The groups infected with *T. congolense* (immunized or unimmunized) were significantly different from their controls infected with only *H. polygyrus* (U=0, P=0.003 for immunized group at 28 DAI and U=0, P=0.0001 at 14 DAI for the unimmunized).

Infections with *H. polygyrus* resulted in rapid and transient increases in the circulating neutrophils. Mice infected with *T. congolense* alone did not produce any substantial change (Figure 9.9b). These changes were not affected by the immune status of the mice to *H. polygyrus*.

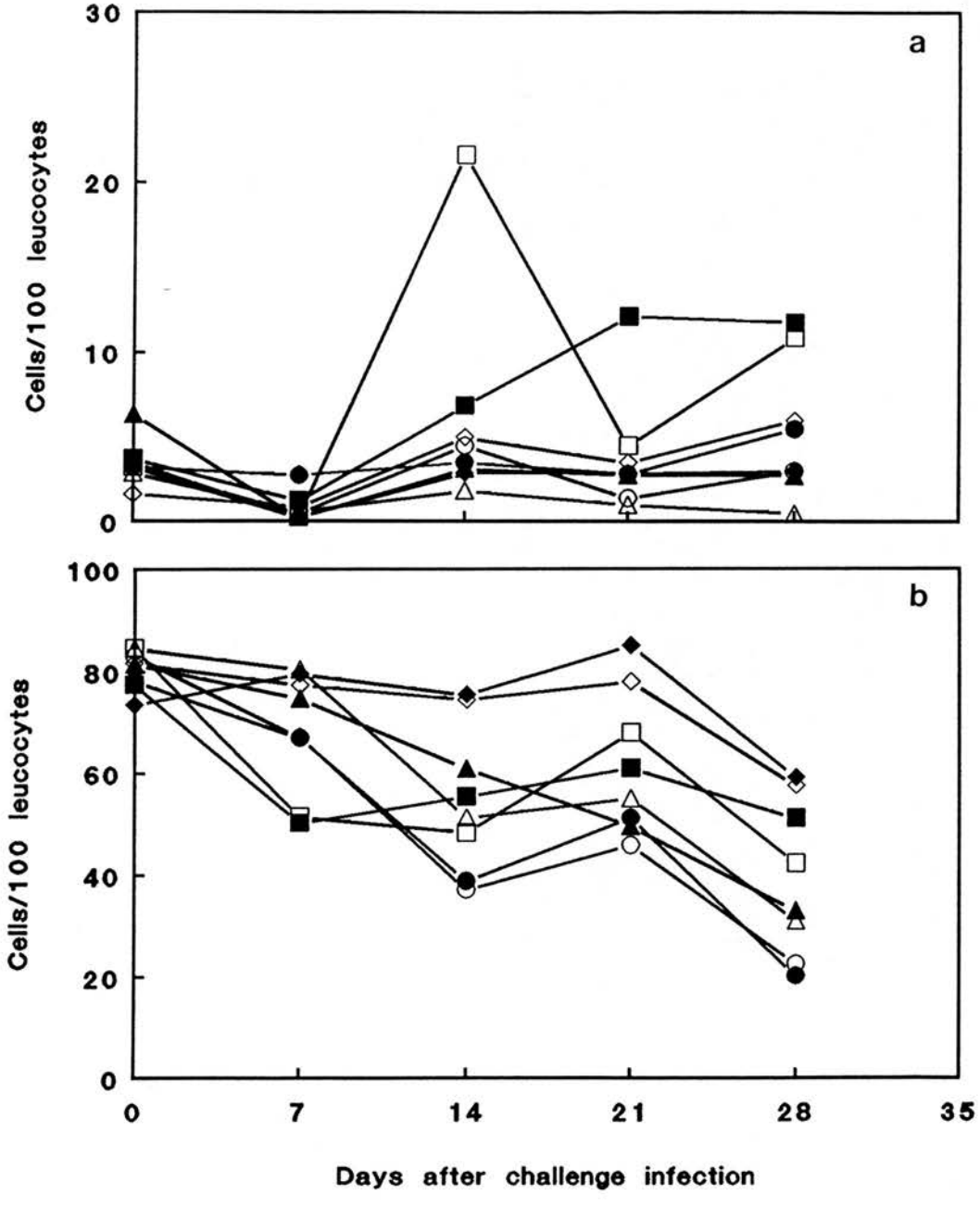

FIGURE 9.8 The mean eosinophil (a) and lymphocyte (b) counts of immunized (●, ■, ▲, ◆) or unimmunized (○, □, △, ◇) mice, uninfected (◆, ◇) or challenged with *T. congolense* alone (▲, △) or *H. polygyrus* alone (■, □) or together with *T. congolense* (●, ○)

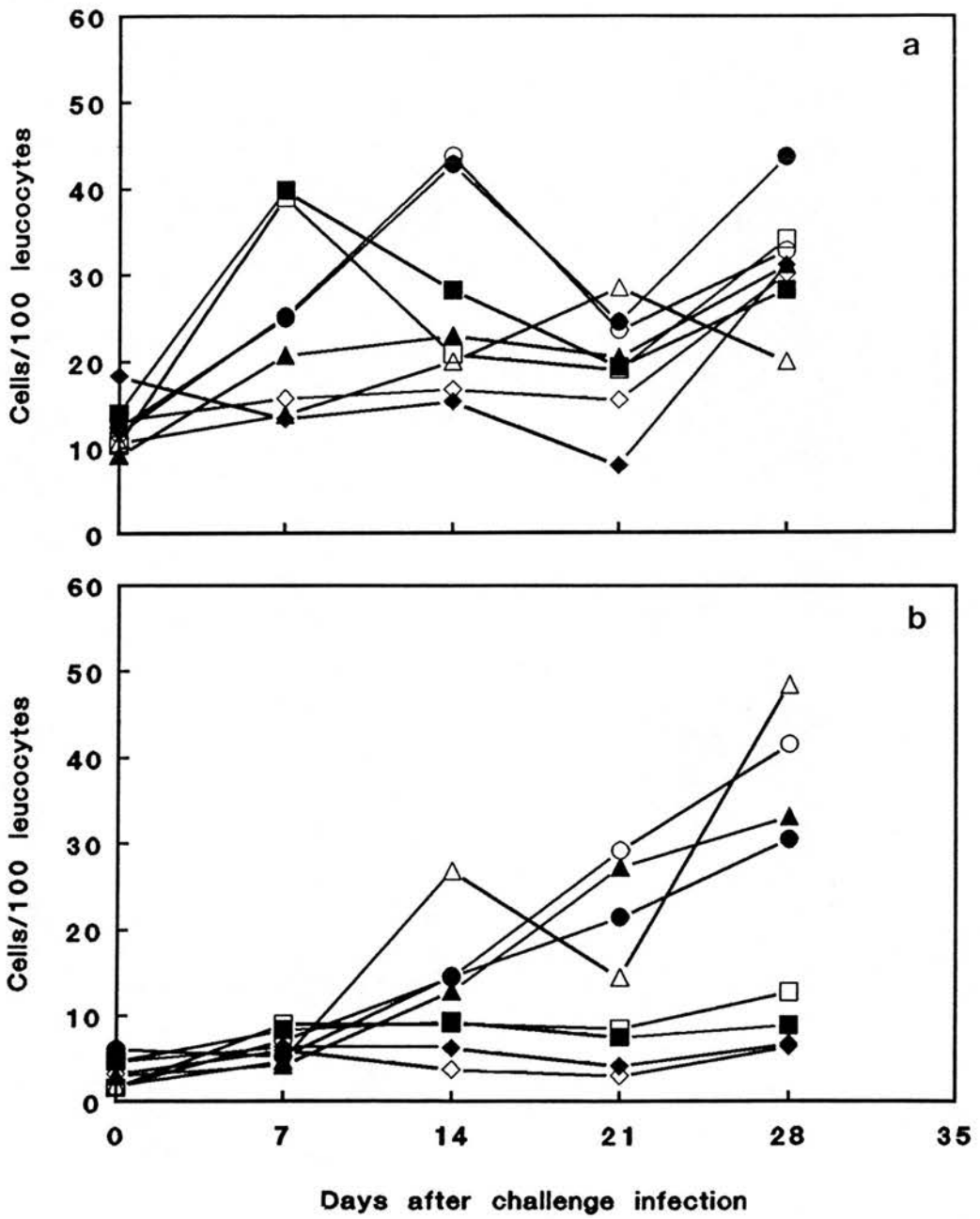

FIGURE 9.9 The mean monocyte (a) and neutrophil (b) counts of immunized (●,■,▲,◆) or unimmunized (○,□,△,◇) mice, uninfected (◆,◇) or challenged with *T. congolense* alone (▲ , △) or *H. polygyrus* alone (■, □) or together with *T. congolense* (●, ○)

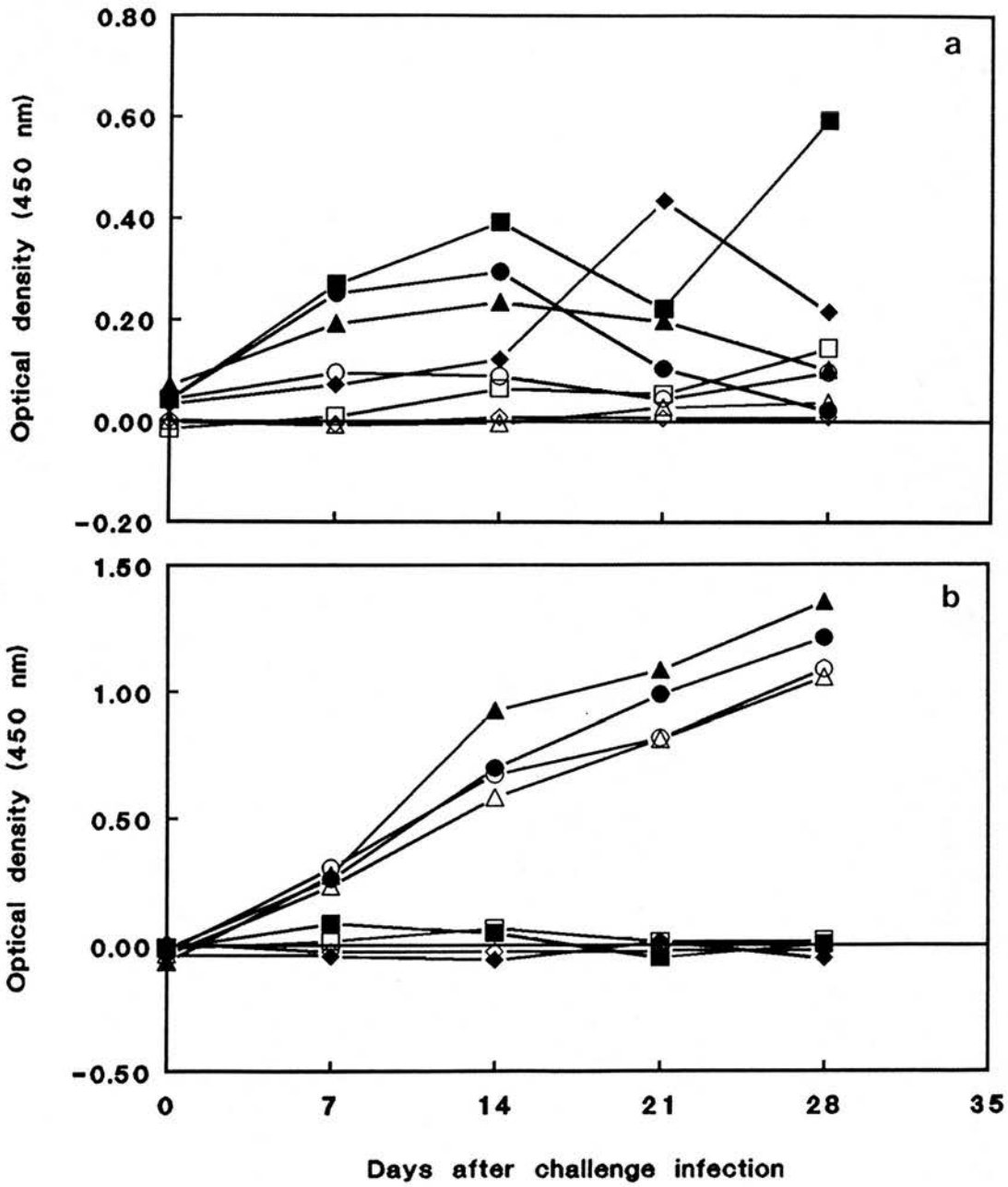

FIGURE 9.10 The antibody response of immunized (●,■,▲,◆) or unimmunized (○,□,△,◇) mice, uninfected (◆,◇) or challenged with *T. congolense* alone (▲,△) or *H. polygyrus* alone (■,□) or together with *T. congolense* (●,○) to *H. polygyrus* (a) or *T. congolense* (b) antigens

Immunized mice infected with *H. polygyrus* alone or conjointly with *T. congolense* showed early rise in response to *H. polygyrus* antigens. While immunized mice continued to maintain high levels of response, those conjointly infected showed a falling level of response to a level significantly lower (U=0, P=0.004), at 28 DAI, than its control infected with *H. polygyrus* alone (Figure 9.10a).

An increasing antibody response was observed in all mice infected with *T. congolense*, irrespective of their immune status or infection with *H. polygyrus* (Figure 9.10b).

9. 4 DISCUSSION

This study confirmed previous observations that mice conjointly infected with *H. polygyrus* and *T. congolense* were severely compromised and clearly demonstrated that this interactive infection markedly depressed the homologous protective response of mice immunized against *H. polygyrus* infection. Loss of the homologous immunity to *H. polygyrus* was associated with a decline in antibody responses to *H. polygyrus* antigens as well as a depressed eosinophilic response.

Dual *H. polygyrus/T. congolense* infection was associated with a severe impairment in acquired immunity to *H. polygyrus* infection, which resulted in a substantial increase in the worm burden of immunized mice. A similar observation was made in Saanen x Galla goats, known to be resistant to *H. contortus*, in which the innate resistance was overcome by a *T. congolense* infection, resulting in more severe clinical effects than in animals infected with *H. contortus* alone (Griffin *et al.*, 1981a). Nevertheless, mice immunized against *H. polygyrus* challenge survived better than those that were not immunized. The pattern of mortality of unimmunized mice infected with *T. congolense* in the present experiment was unusual and is difficult to explain.

The similar fall in PCV values observed in groups of mice infected with either *T. congolense* alone or with both parasites compared with the transient anaemia in mice infected with *H. polygyrus* suggests that, as in earlier experiments,

T. congolense was mainly responsible for lowering the PCV in the conjoint infections and that the immune status of the mice against *H. polygyrus* did not affect this haematological parameter.

As in previous experiments, the *T. congolense* parasitaemias in the different experimental groups were indistinguishable, confirming again the unsuitability of using this parameter in interpreting the effect of interactive response in the present model. As expected, the worm egg count in the faeces from immunized mice was lower than that those not immunized against *H. polygyrus*, but the counts in the faeces from those with dual infection were consistently higher than that from those with *H. polygyrus* infection alone especially during during the first the three weeks of infection. The slow rise in the worm egg count in the faeces from the immunized control with *H. polygyrus* challenge may be a reflection of stunted development associated with immunity to the nematode which was particularly apparent in the female worms.

Although studies in goats and cattle indicated that dual infection of *T. congolense* and *H. contortus* resulted in an enhanced loss in weight of the animals (Griffin *et al.*, 1981a; Kaufmann *et al.*, 1992), the live weight gains of mice with dual *T. congolense/H. polygyrus* infection and those with single *H. polygyrus* infection or uninfected mice did not differ up to 21 DAI. The relative increase in weight thereafter by all mice infected with *T. congolense* was probably due to the gross hyperplasia of the lymphoid organs such as the spleen as exemplified by the weight of the spleen post mortem, which made up to 15% of the weight of the mice.

In mice and rats, *T. brucei* has been shown to cause reduced local and systemic antibody responses to *N. brasiliensis* (Urquhart *et al.*, 1973; Wedrychowicz, Maclean and Holmes, 1984). The present experiment has similarly showed that *T. congolense* infection reduces systemic antibody responses to *H. polygyrus* infection in mice. Although the mechanisms by which this is achieved are not certain, it has been suggested that immunosuppression in the presence of

living trypanosomes may be mediated through a B-lymphocyte defect (Murray *et al.*, 1974c). Trypanosome infection in mice is also known to cause a marked increase in the numbers and activities of the cells of the mononuclear phagocytic system in the liver, lymph nodes, spleen, bone marrow and also in non-fixed macrophages of all tissues (Murray *et al.*, 1974b). Such activated macrophages have been shown to inhibit T-cell responses *in vivo* and *in vitro* (Borowy *et al.*, 1990). The role of suppressor macrophages in trypanosome-induced immunosuppression has been confirmed by experiments in which depletion of the monocyte-macrophage population of cells derived from the lymph nodes or peripheral blood of *T. congolense*-infected cattle or *T evansi*-infected sheep effectively abrogated previously observed immunodepression to a T-cell mitogen (concanavalin A) stimulation (Flynn and Sileghem, 1991; Onah, 1992). The increased numbers of circulating monocytes in the present study may have been associated with the generation of suppressor macrophages.

Furthermore, although $CD4^+$ T-cells (T_H2) have been known to regulate host protective immunity in *H. polygyrus* infections (Urban *et al.*, 1991a; Monroy and Enriquez, 1992), trypanosome infections can directly induce a decrease in the number of $CD4^+$ cells or indirectly through an enhanced production of IFN-γ by the $CD8^+$ cell (Onah, 1992) which may lead to an inhibition of $CD4^+$ cell proliferation. Thus depression of protective responses against *H. polygyrus* in the present study may be by *T. congolense*-induced processes which lead to inhibition of the provision of antigen-specific help to B-cells.

Studies with *T. colubriformis* in guinea pigs and sheep have suggested that eosinophilia is a measure of the immune mediated response to helminth infection (Handlinger and Rothwell, 1981; Dawkins, Windon and Eagleson, 1989). The eosinophilia which has also been identified as an important component of host protective immunity against *H. polygyrus* (Cypess, 1972; Hurley and Vadas, 1983) was specifically suppressed in immune mice additionally infected with *T. congolense*. If, as suggested by Pritchard *et al.* (1983), IgG_1 dependent eosinophil

killing constitutes the main form of anti-parasite immunity in immune animals, the abrogation of immunity to *H. polygyrus* in this study may have resulted from the *T. congolense*-induced suppression of both the antibody and eosinophilic response during the challenge infection.

In conclusion, this study has shown that *T. congolense* suppressed both the humoral and cellular protective responses to *H. polygyrus* infection in immune mice. How these effects are induced are not yet fully understood.

CHAPTER TEN

THE EFFECT OF CONJOINT *HELIGMOSOMOIDES POLYGYRUS* AND *TRYPANOSOMA CONGOLENSE* INFECTION IN 'TO' MICE IMMUNIZED AGAINST SECONDARY *H. POLYGYRUS* INFECTION BY AN ABBREVIATED LARVAL INFECTION

10.1 INTRODUCTION

Mice previously immunized by an anthelmintic terminated adult *H. polygyrus* infection lost almost all the protection they had acquired against a homologous challenge if this was administered simultaneously with *T. congolense* (Chapter 9). It is not as yet understood whether this was due to an inferior initial protective response mounted by mice being completely overwhelmed by the immunodepressive influence of the trypanosomes or by a complete blockage of immune effector mechanism by trypanosomes.

The main purpose of the experiments described in the present chapter was to determine the effects of *T. congolense* infection on the development and maintenance of homologous *H. polygyrus* resistance in mice immunized (primed) by abbreviation of larval infection which had previously been shown to give almost solid immunity (Chapter 7). In addition, the effects of the time of trypanosome infection, whether this was given simultaneously with or before the challenge *H. polygyrus* infection were assessed, to elucidate whether an earlier trypanosome infection compromises animal to the same extent as a simultaneous infection with both parasites.

10.2 MATERIALS AND METHODS

Groups of eight TO mice each were either unimmunized or immunized by infection with 500 L_3 of *H. polygyrus* followed by treatment with ivermectin (20mg/kg body weight) six days after infection (Chapter 7). When the immunizing infection was terminated all the experimental mice, including those without any previous infection, were treated with ivermectin. Twenty one days after the anthelmintic treatment, immunized and unimmunized mice were infected with the desired combinations of standard doses of *H. polygyrus* (500 L_3) and *T. congolense* (104 blood stream forms of TREU 1881) or left as uninfected control as detailed in Table 10.1.

Daily worm faecal egg counts and the *T. congolense* parasitaemias were determined for *H. polygyrus* and *T. congolense* infected mice respectively. From

the start of the secondary parasite infection, the weekly PCVs and live weights of each mouse were also determined. The antibody responses of individual mice to *H. polygyrus*- and *T. congolense*-derived antigenic preparations were also determined by ELISAs performed on sera collected weekly.

The survival of the mice was recorded daily and post mortem worm counts (males and females) and carcass (without the viscera) weights were obtained. The initial eviscerated carcass weight of each mouse was estimated from a calculated relationship between the mean body weight and the mean eviscerated carcass weight of five randomly selected mice that were killed at the start of the experiment. The weight gains or losses for the mice were calculated by subtracting the initial eviscerated carcass weights from those taken 30 days after the secondary parasite infection, when the experiment was terminated.

TABLE 10.1 Experimental groupings and schedule of infections of mice previously immunized by termination of larval infection or not immunized against *H. polygyrus* challenge with *H. polygyrus* (HP) alone or *T. congolense* (TC) alone or both parasites together.

Group	Immunization	Days of challenge infections 0	10
ITH/0	+	HP+TC	-
IH/0	+	HP	-
IT	+	TC	-
UH/0	-	HP	-
IC	+	-	-
UC	-	-	-
ITH/10	+	TC	HP
IH/10	+	-	HP
UTH/10	-	TC	HP

10.3 RESULTS

The clear differences between the faecal worm egg counts of immunized and unimmunized mice are shown in Figure 10.1. Counts in all immunized groups were consistently similarly depressed. All the unimmunized mice that were infected with *T. congolense* 10 days before *H. polygyrus* died within seven days of the nematode infection, thus no egg count was obtained from this group.

Parasitaemias in the mice infected with *T. congolense* ran similar courses, mice being parasitaemic from 5 DAI and rapidly rising to a plateau at 10 DAI (Figure 10.2a).

All the uninfected mice and those infected with *H. polygyrus* alone survived till the end of the experiments. Significantly more (U=12.0, P=0.0379) immunized mice conjointly infected with both parasites survived in the group infected simultaneously than the comparable group in which *H. polygyrus* was superimposed on a 10 day old *T. congolense* infection. Although all the mice in which *T. congolense* preceded *H. polygyrus* died before the experiment was terminated, the decline in the numbers of survivors in the immunized group was more prolonged than the unimmunized group (Figure 10.2b).

Mice not infected with any parasite and those infected with *H. polygyrus* alone showed minimal fluctuations in their PCVs. The PCV in mice with a simultaneous dual infection and those with only the *T. congolense* infection dropped by 12.6% and 19.4% at 14 DAI respectively and remained at this level or recovered slightly. When the *T. congolense* infection preceded the challenge or initial *H. polygyrus* infection, there was a drastic reduction in the PCV of both immune and unimmunized mice after infection with the helminth, which was more marked in the unimmunized (Figure 10.3a).

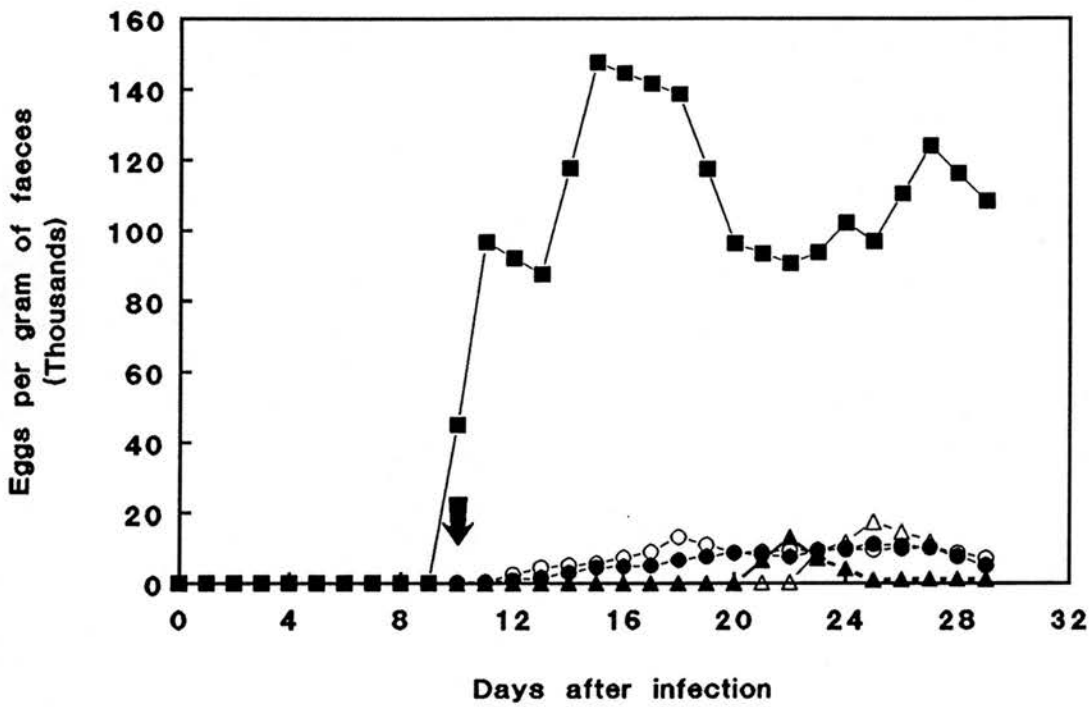

FIGURE 10.1 Faecal worm egg counts from unimmunized mice infected with *H. polygyrus* alone (■) or immunized mice infected with *H. polygyrus* alone (●) or simultaneously with *T. congolense* (○) and counts from immunized mice infected with *H. polygyrus* alone (▲) or conjointly infected with *T. congolense* 10 days before the nematode infection (△). Arrow indicates the time of *H. polygyrus* infection of ITH/10 and IH/0

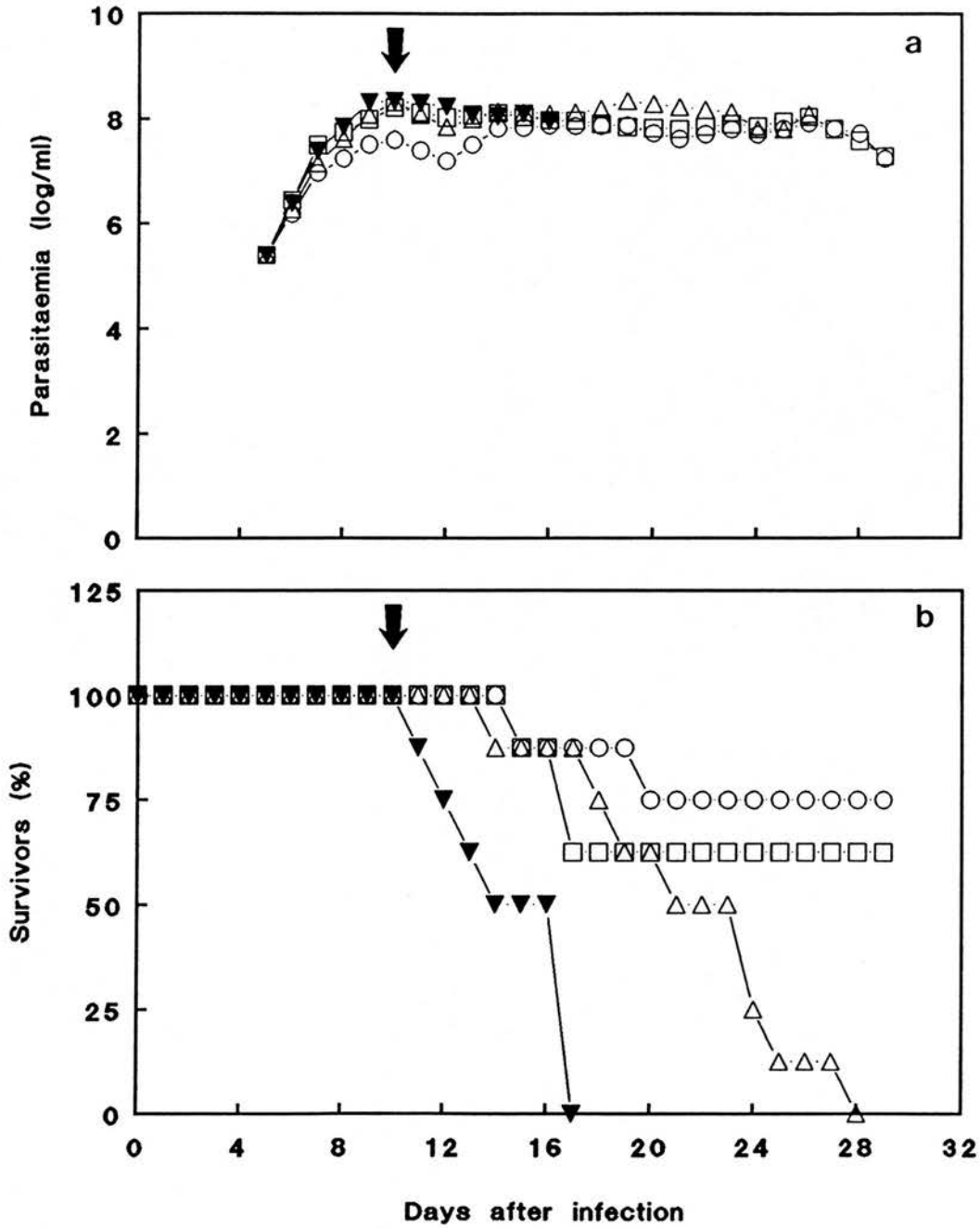

FIGURE 10.2 The mean *T. congolense* parasitaemia (a) and mortality (b) of immunized mice infected with *T. congolense* alone (□) or simultaneously infected (●) or infected 10 days later (△) with *H. polygyrus* and the parasitaemia (a) and mortality (b) of unimmunized mice in which *H. polygyrus* was superimposed on a 10 day old *T. congolense* infection (▼). The arrow indicates time of infection of ITH/10 and UTH/10 with *H. polygyrus*. For the values from individual mice see Appendix Tables

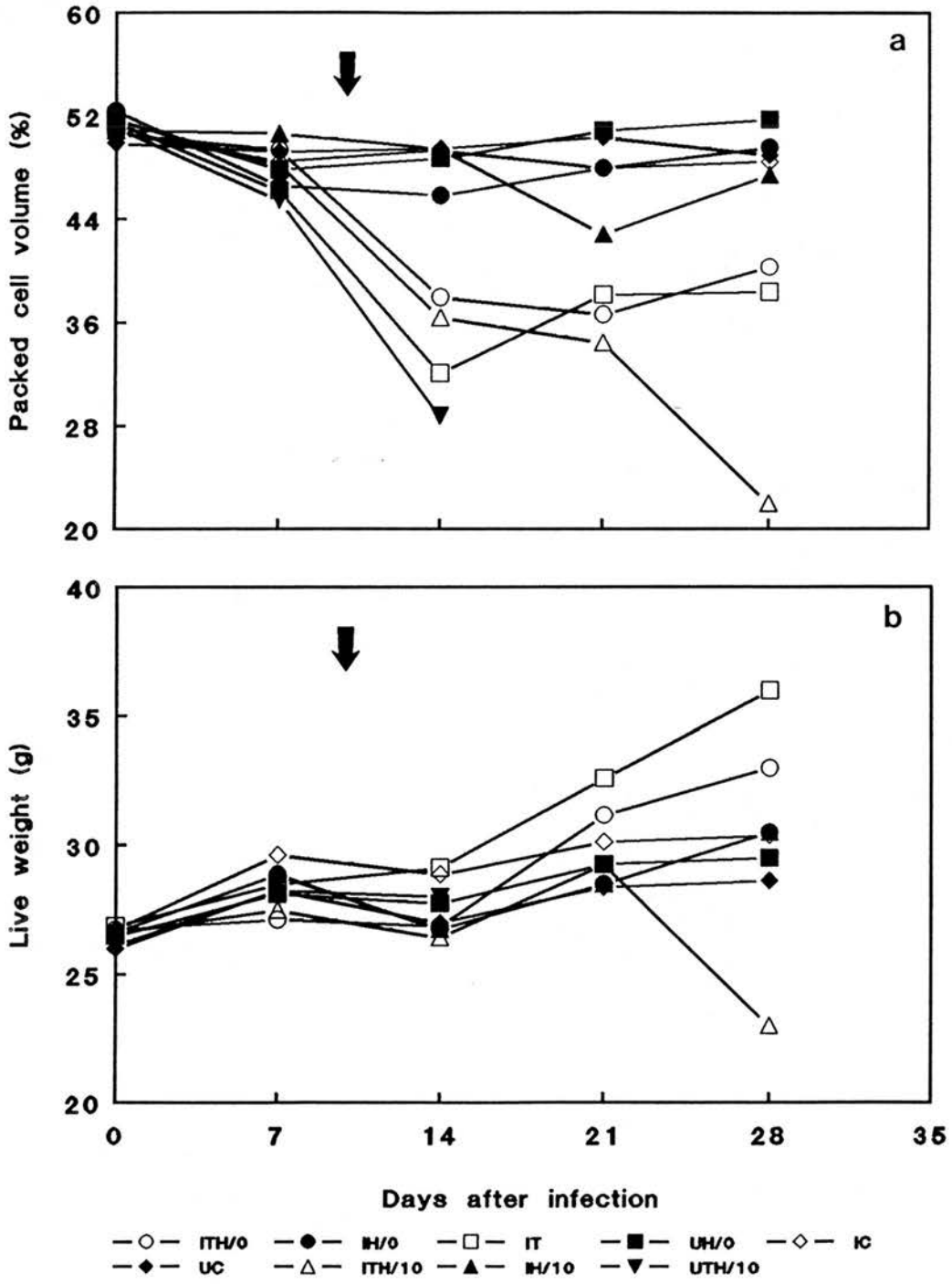

FIGURE 10.3 The mean PCVs (a) and weights (b) of immunized or unimmunized mice not infected or infected with *H. polygyrus* alone or together with *T. congolense*. The arrow indicates time of infection of ITH/10 and UTH/10 with *H. polygyrus*. For the values from individual mice see Appendix Tables

Mice with *T. congolense* infected tended to gain more live weight than others, especially after 14 DAI (Figure 10.3b). The mean live and eviscerated carcass weights of the representative mice that were killed at the beginning of the study were 24.7g and 18.7g (i.e. 75.7% of the live weight) respectively. The difference between eviscerated carcass weights at the start, estimated to be 75.7% of the live weight, and the end of the experiments show that mice with dual infections suffered weight losses instead of weight gains (Figure 10.4).

The unimmunized infected control mice (UH/0) had a mean worm burden of 286.9, i.e. 57.4% establishment. The worm burdens of immunized mice that were challenged with *H. polygyrus* alone (IH/0) or simultaneously with *T. congolense* (ITH/0) on day zero were significantly lower (U=0, P=0.0002 for IH/0) than the unimmunized control, amounting to 91.2% and 84.3% protection respectively (Figure 10.5). A rough estimation of the protection in immunized mice in which *T. congolense* preceded *H. polygyrus* infection (ITH/10) obtained from the only surviving mouse was 53.3%. This was based on the worm burden of UH/0.

A similar group challenged with *H. polygyrus* alone (IH/10) which had a mean worm burden of 20.4, equivalent to 92.9% protection (Figure 10.5).

There was a rapid rise in the antibody response to *H. polygyrus*-derived antigens in the sera of immunized mice following challenge with *H. polygyrus* alone and by day 28, the antibody titres in IH/0 were clearly higher (U=6, P=0.01) than those of a similar group (ITH/0) conjointly infected with *T. congolense* (Figure 10.6a). The unimmunized mice in which *T. congolense* infection preceded *H. polygyrus* infection (UTH/10) had not responded before their death.

The response to *T. congolense* antigens by immunized mice infected with *T. congolense* alone (IT) or simultaneously with *H. polygyrus* (ITH/0) rose rapidly and proportionately as the infection progressed (Figure 10.6b). Although there was a similar initial rise in the antibody titres in immunized or unimmunized mice in which *T. congolense* preceded *H. polygyrus*, the titres in ITH/10 remained almost

unaltered from 14 DAI (i.e. 4 days after *H. polygyrus* infection). Uninfected mice and those infected with *H. polygyrus* alone were unresponsive to the *T. congolense* antigen (Figure 10.9b). At 28 DAI, ITH/10, antibody body titre to *T. congolense*-derived antigens remained reduced.

10.4 DISCUSSION

The results presented in this chapter again confirmed that conjoint *H. polygyrus* and *T. congolense* infection is detrimental to the TO mice. More drastic effects, including increased mortality and weight losses and interference with acquired immunity against *H. polygyrus*, were associated with infections in which *T. congolense* infection preceded *H. polygyrus* infection.

Dual infection with both parasites did not have any apparent effect on the the EPG of immune mice, this being a reflection of the similar worm burdens in these mice (Figure 10.5). A true comparison of the worm burdens in the controls and in the groups in which *T. congolense* infection preceded *H. polygyrus* infection is impossible because all but one of the mice with such dual infections died before post mortem worm counts were performed. The only evidence from the single surviving mouse in group ITH/10 suggests that up to 40% of the expected protection against *H. polygyrus* was lost when *T. congolense* infection preceded the nematode infection.

Studies with other host-parasite systems have shown that some haemoprotozoans are capable of interfering with the development of resistance to a homologous helminth challenge. For instance, both acute malaria (*P. berghei*) and trypanosome (*T. brucei*, TREU 792) infections initiated at the same time as *T. muris* infection suppressed the immune expulsion of the challenge infection (Phillips *et al.*, 1974). Similar effects due to *T. brucei* were also observed in mice with *E. revolutum* infection (Christensen *et al.*, 1984). In the present study, although humoral antibodies against *H. polygyrus* antigens in dual infections in

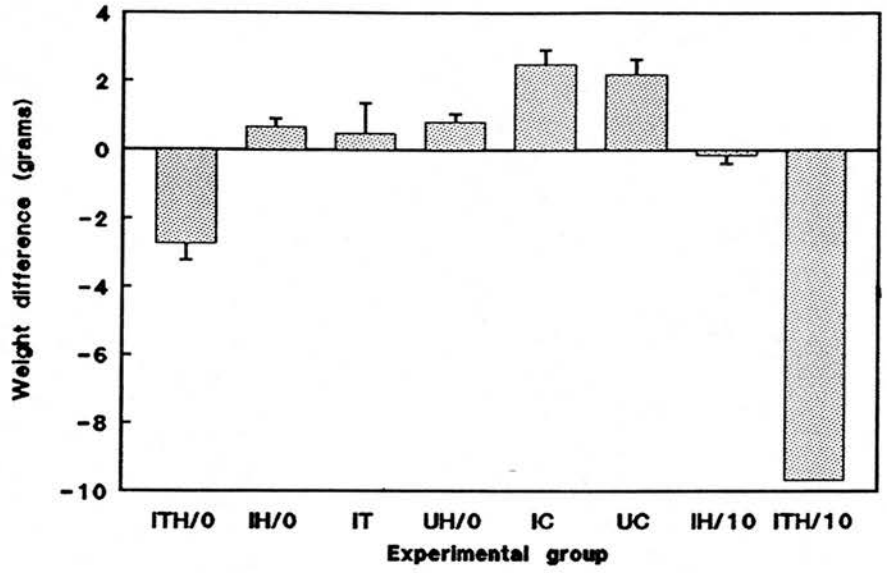

FIGURE 10.4 The mean (±SEM) change in the eviscerated carcass weight of immunized or unimmunized mice not infected or infected with *H. polygyrus* alone or *T. congolense* alone or with both parasites. For values for individual mice see Appendix Tables

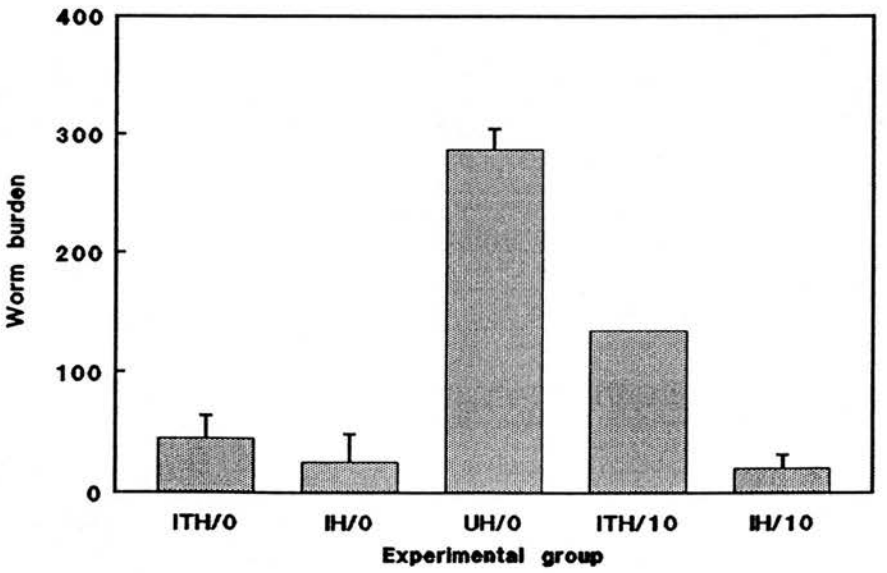

FIGURE 10.5 The mean (±SEM) *H. polygyrus* burden from immunized or unimmunized mice infected with *H. polygyrus* alone or together with *T. congolense*. For values for individual mice see Appendix Tables

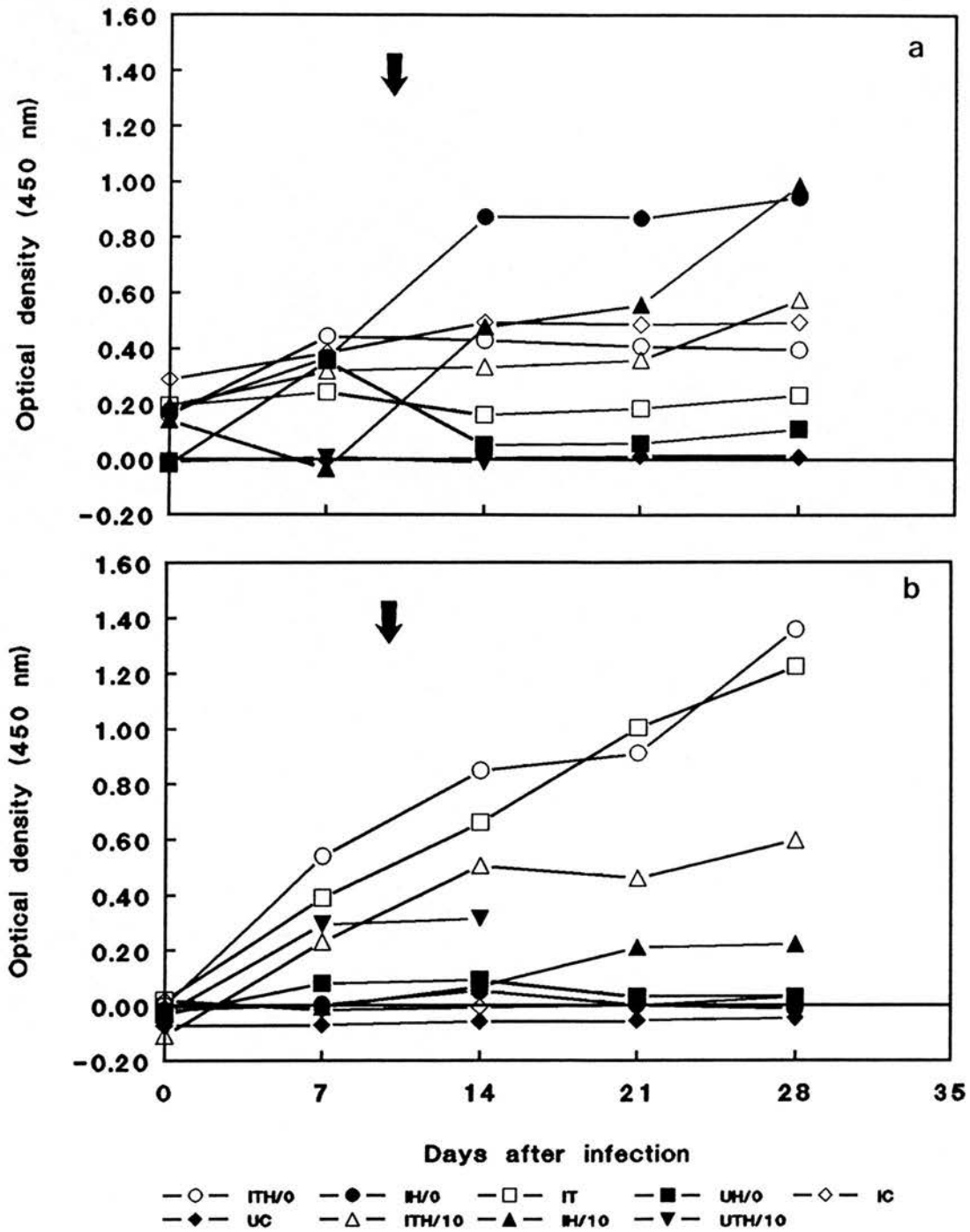

FIGURE 10.6 The mean antibody titres against *H. polygyrus*-derived antigens (a) or *T. congolense*-derived antigens (b) by immunized or unimmunized mice not infected or infected with *H. polygyrus* alone or together with *T. congolense*. The arrow indicates time of infection of ITH/10 and UTH/10 with *H. polygyrus*. For the values from individual mice see Appendix Tables

immune mice remained at a similar level to those in their respective unchallenged control, those simultaneously infected with *T. congolense* and the *H. polygyrus* challenge retained substantial protection but the response in the mice in which *T. congolense* preceded the *H. polygyrus* secondary infection was markedly impaired. This suggests that *T. congolense*-induced interference with acquired immunity to *H. polygyrus* infection in mice may be influenced, among other things, by the duration of the *T. congolense* infection prior to the challenge *H. polygyrus* infection.

Although *H. polygyrus* has been shown to depress the immune response to heterologous antigens (Chowaniec *et al.*, 1972; Shimp *et al.*, 1975; Ali and Behnke, 1983), there have been no reports of *H. polygyrus* enhancing the pathogenicity of a blood protozoan. Bell *et al.* (1984a) reported that, although concomitant infection with *Trichinella spiralis* in the mouse increased the maximum *Trypanosoma musculi* parasitaemia by two- to four-fold, regardless of the degree of resistance of the murine strain to either *T. musculi* or *T. spiralis*, *H. polygyrus* did not promote a *T. musculi* parasitaemia over the level of a single infection. In the present study, although the levels of *T. congolense* parasitaemia in singly and dually infected mice were identical, there was clear potentiation of the pathogenicity of *T. congolense* as indicated by the clinicopathological parameters including mortality, eviscerated carcass weights and serum antibody response, especially when *H. polygyrus* infection was superimposed on a preexisting protozoan infection. Kaufmann *et al.* (1992) also observed that, in N'Dama cattle, *Haemonchus contortus* did not influence *T. congolense* parasitaemia but drastically increased weight losses and the mortality of animals in which trypanosomes were present prior to infection with the nematode, this being the most harmful combination. In the present study, the pathological effects of *T. congolense* on the mice may have been exacerbated by the disruptive effects of the *H. polygyrus* larvae, whose development in the intestine produces most of the

pathological lesions in heligmosomoidosis (Spurlock, 1943; Baker, 1955; Liu, 1965a).

The synergistic effects of *H. polygyrus* on the *T. congolense* infection might have also arisen from immunodepression of the host's response to the protozoan caused by *H. polygyrus*, as demonstrated by the decreased anti-*T. congolense* serum antibody titres in those mice which received *H. polygyrus* 10 days after the *T. congolense* infection. The mechanisms whereby such immunodepression is mediated are not understood. However, it has been demonstrated that both the larval and adult stages of *H. polygyrus* secrete immunomodulatory factor(s) (Behnke *et al.*, 1983, Behnke, 1987; Losson *et al.*, 1985; Monroy, Dobson and Adams, 1989), which facilitate their own survival in the gastrointestinal tract. These factors may also be implicated in the suppression of heterologous immunity. Both *in vitro* and *in vivo* studies have shown that soluble antigens in a homogenate of *H. polygyrus* depress the response to heterologous sheep RBC in mice (Pritchard *et al.*, 1984; Crawford, Behnke and Pritchard, 1989). Such non-specific immunodepression has been attributed to the generation of suppressor cells (Crawford *et al.*, 1989; Monroy *et al.*, 1989).

In conclusion, *T. congolense* does not completely block the strong acquired resistance induced by abbreviated *H. polygyrus* larval infection in TO mice but it is capable of interfering with these protective responses if administered prior to *H. polygyrus* challenge infection. In these mice, *H. polygyrus* on the other hand produces synergistic pathological effects on the protozoan infection.

CHAPTER ELEVEN

CONCLUDING REMARKS

The present study has demonstrated that, although *Trypanosoma congolense* can now be grown *in vitro* to produce large numbers of infective metacyclic forms, reproducible parasitological results could only be obtained by intraperitonial infection of female TO mice with stabilated blood stream forms. These TO mice also tolerated relatively large doses of *Heligmosomoides polygyrus* infection without any overt sign of disease making this host-parasite system an excellent model for studying chronic gastro-intestinal helminthosis of mammals (Bartlett and Ball, 1972; Monroy and Enriquez, 1992).

Experimental host-parasite systems have often been used as models for infections in man and domestic animals in order to gain insight into the epidemiological, pathological and immunological mechanisms of natural infections. However, the chosen experimental system must closely resemble the natural infections if meaningful deductions, interpretation and application of results are to be achieved. For instance, nematodes such as *Trichinella spiralis* in mice and *Nippostrongylus brasiliensis* in rats which produce short and host-abbreviated infections (Crandall and Crandall, 1972; Urquhart *et al.*, 1973), although useful for some purposes, are not appropriate for studying chronic nematode infections. The work described in this thesis thus demonstrates that, the current host-parasite system is suitable for studying aspects of concurrent trypanosome and chronic nematode infections similar to the few available studies in ruminants (Griffin *et al.*, 1981a,b; Kaufmann *et al.*, 1992).

In relation to dual infections, this work has made the following principal new observations:

1. Except when *T. congolense* was superimposed on a 10-day old *H. polygyrus* infection, mice conjointly infected with *T. congolense* during a primary *H. polygyrus* infection were severely compromised, resulting in enhanced mortality. The synergistic pathogenic effects of dual infections in mice were particularly marked when *T. congolense* infection preceded infection with the nematode.

2. *Trypanosoma congolense* infection depressed the immune (cellular and humoral) responses which normally occur in mice after primary *H. polygyrus* infection.

3. The protozoan infection either reduced or totally inhibited immunity against a challenge *H. polygyrus* infection.

4. In spite of the suppressed immune response in mice against *H. polygyrus* in ELISA, there was no apparent influence on the growth of the nematode, except that female worms from mice that were infected with *T. congolense* when the nematode infection was 10-days old were stunted 30 days after infection.

These observations, taken together with field reports relating to trypanosome and nematode infections in animals (Griffin *et al.*, 1981a,b; Kaufmann *et al.*, 1992; Fakae and Chiejina, 1993) suggest that, conjoint infections with these parasites produce deleterious synergistic interactions which affect productivity and even cause deaths. Although there was a seemingly antagonistic effect on the growth of 10-day old *H. polygyrus* when conjointly infected with *T. congolense*, it is difficult to predict the outcome following prolonged nematode infection. Synergistic and antagonistic interactions may both develop in a given concurrent infection as demonstrated by the initial reduced establishment followed by delayed expulsion of *N. brasiliensis* during a joint infection with *H. polygyrus* (Bruna and Xenia, 1976). In the present model, clinicopathological observations, rather than parasitological parameters, clearly showed the synergistic interactions.

Although the protective responses against homologous challenge in mice immunized by abbreviation of *H. polygyrus* adult infection were completely lost as a result of concurrent infection with *T. congolense*, the stronger protection in those immunized by an abbreviated larval infection was merely reduced. These observations suggest that animals with a strong immunity to gastrointestinal nematodes may largely overcome the suppressive influence of the trypanosome. It is probable that there is a qualitative difference in the immunity induced by the two immunization procedures. Considerable progress has been made towards the

production of commercial vaccines for the control of gastrointestinal nematodes of domestic animals over the last few years (Emery and Wagland, 1991). The present observation of markedly depressed immunity by *T. congolense* in mice with medium protection against *H. polygyrus* should provide some caution that only vaccines with the highest protection may be useful in areas endemic for African trypanosomes.

Ruminants have been shown, both experimentally and under field conditions to acquire resistance to gastrointestinal nematodes (Chiejina and Sewell, 1974a,b; Chiejina, 1986; Douch, 1989; Pomroy and Charleston, 1989a; Rothwell, 1989; Dobson, Waller and Donald, 1990a,b; Emery, McClure, Wagland and Jones, 1992a,b; McClure, Emery, Wagland and Jones, 1992) and this resistance may be responsible for the low grade worm burdens characteristic of these infections in the field (Fakae, 1990b). Enhanced resistance through abbreviation of a primary nematode infection after the first week of grazing an infected pasture at the beginning of the grazing season in tropical areas with a long dry season might help combat such problems of synergistic interactions during natural infections of nematodes and trypanosomes in ruminants. This possibility requires confirmation.

The complex influence of ecological factors on the pattern and frequency of concurrent infections, makes extrapolation from experimental studies to naturally occurring infections difficult (Christensen *et al.*, 1987). However, valuable information on the nature and characteristics of heterologous interactions between a parasite and its mammalian host has been obtained but further investigations are required to fully elucidate the disease-related consequences of concomitant infections with salivarian trypanosomes and chronic nematode infections of mammals and especially of ruminants. This work should be extended to include studies using both sexes of different strains of mice including those with a predetermined responder status to both parasite infections. Similar controlled studies with sheep, goats and cattle using naturally occurring parasites, and under both laboratory and field conditions, are also necessary.

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APPENDIX ONE

TABLES OF VALUES FROM INDIVIDUAL MICE

TABLE A4.1

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.1)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
7	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
8	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
9	4.6	4.6	5.3	5.3	4.6	4.6	4.83	0.1	6
10	4.6	4.6	5.3	5.3	4.6	4.6	4.83	0.1	6
11	4.6	4.6	5.3	5.3	4.6	4.6	4.83	0.1	6
12	4.6	4.6	7.3	4.6	4.6	4.6	5.05	0.4	6
13	4.6	4.6	8.9	4.6	4.6	4.6	5.32	0.7	6
14	4.6	4.6	9.2	5.3	4.6	4.6	5.49	0.7	6
15	4.6	5.6	8.7	5.3	4.6	4.6	5.57	0.6	6
16	7.3	5.6		5.6	4.6	6.6	5.94	0.4	5
17	8.5	5.9		5.9	5.3	6.9	6.50	0.5	5
18	9.0	8.0		8.0	5.6	8.4	7.78	0.5	5
19		9.2		9.3	8.7	9.0	9.08	0.1	4
20		9.3		9.4	9.3	9.3	9.33	0.0	4
21		9.3		9.3	9.3	9.3	9.32	0.0	4
22		9.2		9.4	9.3	9.4	9.33	0.0	4
23		9.1		9.4	9.4	9.3	9.29	0.1	4
24		9.1		9.1	9.1	9.3	9.17	0.1	4
25					8.2		8.20	0.0	1
26					8.2		8.20	0.0	1
27					7.8		7.80	0.0	1
28									0

TABLE A4.2

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^5 organisms (Experiment 4.1)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
7	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
8	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
9	4.6	5.3	4.6	4.6	4.6	4.6	4.72	0.1	6
10	4.6	5.3	4.6	4.6	4.6	4.6	4.72	0.1	6
11	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
12	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
13	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
14	8.5	9.1	9.0	4.6	8.4	8.7	8.05	0.6	6
15	9.3			7.1	9.1		8.49	0.6	3
16				9.2			9.16	0.0	2
17				9.2			9.24	0.0	2
18				8.5			8.50	0.0	2
19				8.4			8.41	0.0	2
20				8.3			8.25	0.0	2
21									0

TABLE A4.3

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
2×10^3 organisms (Experiment 4.1)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
7	4.6	4.6	5.3	4.6	4.6	5.3	4.83	0.1	6
8	4.6	4.6	5.3	4.6	4.6	5.3	4.83	0.1	6
9	5.3	6.3	5.6	5.9	5.6	5.6	5.72	0.1	6
10	5.3	5.9	5.6	5.9	5.6	5.6	5.65	0.1	6
11	7.0	7.4	5.6	5.6	5.6	6.3	6.25	0.3	6
12	7.3	7.7	4.6	4.6	4.6	6.6	5.90	0.5	6
13	9.2	7.8	8.7	8.9	8.6	9.1	8.70	0.2	6
14	9.5		9.0	9.2	8.9	9.4	9.18	0.1	5
15			9.3				9.26	0.0	2
16			9.3				9.32	0.0	2
17			8.9				8.86	0.0	2
18			8.6				8.60	0.0	2
19									0

TABLE A4.4

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.2)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0	0	0	0	0	0	0.00	0.0	6
1	0	0	0	0	0	0	0.00	0.0	6
2	0	0	0	0	0	0	0.00	0.0	6
3	0	0	0	0	0	0	0.00	0.0	6
4	0	0	0	0	0	0	0.00	0.0	6
5	0	0	0	0	0	0	0.00	0.0	6
6	0	0	0	0	0	0	0.00	0.0	6
7	0	0	0	0	0	0	0.00	0.0	6
8	0	0	0	0	0	0	0.00	0.0	6
9	0	0	0	0	0	0	0.00	0.0	6
10	0	0	0	0	0	0	0.00	0.0	6
11	0	0	0	0	0	0	0.00	0.0	6
12	0	0	0	0	0	0	0.00	0.0	6
13	0	0	0	0	0	0	0.00	0.0	6
14	0	4.6	0	0	0	0	0.77	0.7	6
15	0	4.6	0	0	0	0	0.77	0.7	6
16	0	4.6	5.3	6.9	0	5.3	3.68	1.1	6
17	0	5.3	6.6	7.6	5.9	5.3	5.12	1.0	6
18	5.6	6.3	7.1	8.0	5.9	5.3	6.37	0.4	6
19	5.3	6.6	8.0	6.9	7.1	4.6	6.42	0.5	6
20	6.6	8.6	7.7	8.0	8.2	4.6	7.29	0.6	6
21	6.9	8.9	5.3	8.3	8.5	4.6	7.08	0.7	6
22	6.9	6.6	8.3	8.5	8.5	4.6	7.24	0.6	6
23	5.3	6.6	8.9	8.9	7.4	4.6	6.93	0.7	6
24	7.3	7.4	8.7	9.0	8.5	5.3	7.70	0.5	6
25	5.3	8.0	9.1	8.9	9.0	6.6	7.81	0.6	6
26	7.1	7.3	9.2	9.2	8.7	6.6	8.02	0.4	6
27	7.8	7.6	4.6	9.0	9.1	7.4	7.59	0.6	6
28	5.3	6.6	5.3	8.5	9.3	7.1	7.01	0.6	6
29	5.6	6.6	5.6	8.6	8.9	7.1	7.07	0.5	6
30	7.2	7.6	6.9	7.2	9.2	7.1	7.52	0.3	6
31	7.1	7.9	8.0	7.4	8.8	6.9	7.68	0.3	6

TABLE A4.5

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^4 organisms (Experiment 4.2)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
7	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
8	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
9	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
10	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
11	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
12	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
13	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
14	0.0	4.6	6.6	4.6	4.6	4.7	4.19	0.8	6
15	0.0	4.6	8.1	4.6	7.5	5.3	5.02	1.1	6
16	7.3	6.6	8.4	6.6	6.9	7.6	7.22	0.3	6
17	7.6	7.3	7.8	6.6	7.3	7.9	7.42	0.2	6
18	7.7	7.3	7.8	7.3	7.3	7.9	7.56	0.1	6
19	8.6	7.3	7.3	7.5	6.9	6.6	7.37	0.3	6
20	8.7	7.3	8.6	8.2	8.4	8.5	8.28	0.2	6
21	8.8	7.4	8.9	8.5	8.7	8.8	8.50	0.2	6
22	8.6	8.4	8.8	8.2	8.5	9.0	8.57	0.1	6
23	8.8	8.5	8.9	8.5	7.8	6.6	8.19	0.3	6
24	8.3	7.9	9.1	9.3	8.3	7.9	8.47	0.2	6
25	4.6	6.9	9.2	8.8	9.1	7.4	7.67	0.7	6
26	4.6	6.9	8.9	8.3	8.7	8.4	7.62	0.6	6
27	7.4	7.5	8.9	7.4	9.3	8.9	8.22	0.3	6
28	7.6	8.1	8.7	7.9	9.1	8.6	8.33	0.2	6
29	9.2	9.0	8.9	8.9	9.2	7.9	8.86	0.2	6
30	9.4	8.9	9.4	8.6	8.8	8.3	8.90	0.2	6
31	9.4	8.4	8.7	7.3	9.3	8.7	8.63	0.3	6

TABLE A4.6

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^5 organisms (Experiment 4.2)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	4.6	0.0	0.0	0.0	0.0	0.0	0.77	0.7	6
2	4.6	0.0	5.3	5.3	0.0	5.3	3.42	1.0	6
3	5.3	5.3	5.3	5.3	0.0	5.3	4.42	0.8	6
4	5.3	5.3	5.3	5.3	0.0	5.3	4.42	0.8	6
5	5.9	5.6	5.3	5.3	5.3	4.9	5.38	0.1	6
6	5.9	5.6	5.3	5.3	5.3	5.3	5.45	0.1	6
7	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
8	4.6	4.6	4.6	4.6	4.6	4.6	4.60	0.0	6
9	6.6	4.6	6.6	6.6	6.6	6.6	6.27	0.3	6
10	6.6	4.6	6.6	6.6	6.6	6.6	6.27	0.3	6
11	7.1	6.6	6.6	6.9	6.9	7.3	6.90	0.1	6
12	7.3	8.2	8.3	8.4	8.2	8.2	8.11	0.1	6
13	7.5	8.5	8.6	8.7	8.5	8.5	8.38	0.2	6
14	7.7	8.8	8.9	9.0	8.8	8.7	8.66	0.2	6
15	8.6	9.1	9.3		9.3	9.4	9.14	0.1	5
16	9.2	9.0	9.2		9.2	9.3	9.20	0.0	5
17	9.5				9.0	9.5	9.34	0.1	3
18	9.4					8.7	9.03	0.2	2
19						8.6	8.60	0.0	1
20									0

TABLE A4.7

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.3)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
7	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
8	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
9	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
10	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
11	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
12	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
13	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0	6
14	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
15	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
16	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
17	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
18	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
19	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
20	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
21	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
22	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
23	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
24	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
25	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
26	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
27	0.0	5.3	0.0	0.0	0.0	0.0	5.30	0	6
28	0.0	5.3	0.0	0.0	5.3	0.0	5.30	0	6
29	0.0	5.3	0.0	0.0	5.3	0.0	5.30	0	6
30	0.0	5.3	0.0	0.0	5.3	0.0	5.30	0	6

Note. Other groups in this experiment were not parasitaemic

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.4)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	8
7	0.0	4.6	0.0	0.0	0.0	4.6	0.0	0.0	1.15	0.7	8
8	0.0	4.6	0.0	0.0	0.0	4.6	0.0	0.0	1.15	0.7	8
9	0.0	4.6	0.0	0.0	0.0	4.6	0.0	0.0	1.15	0.7	8
10	8.2	8.3	8.0	8.2	8.4	8.2	7.9	0.0	7.13	1.0	8
11	8.5	8.6	8.3	8.5	8.7	8.5	8.2	6.9	8.26	0.2	8
12	8.8	9.1	8.8	8.3	9.1	8.7	8.6	7.1	8.56	0.2	8
13	9.1	9.5	9.0	8.7	9.4	8.8	9.0	7.3	8.85	0.2	8
14	9.5	9.4	9.0	9.1	9.4	9.0	9.2	8.9	9.18	0.1	8
15	9.0	9.3	8.7	9.1	9.2	8.5	9.0	8.5	8.91	0.1	8
16			9.6	9.6	9.5	7.7		8.9	9.04	0.3	5
17			9.2	9.6	8.6	7.2		8.8	8.69	0.4	5
18			9.3		8.9	8.2		8.9	8.82	0.2	4
19			9.0		8.9	7.7		8.9	8.61	0.3	4
20			8.9		8.6			9.1	8.89	0.1	3
21			8.9		6.3			7.9	7.70	0.6	3
22			9.2		6.3			8.8	8.10	0.7	3
23			9.3		5.6			9.3	8.06	1.0	3
24			9.1		6.6			9.1	8.26	0.7	3
25			9.0		6.6			8.7	8.11	0.6	3
26					8.1			8.8	8.47	0.2	2
27					8.3			8.2	8.26	0.0	2
28					7.7			4.6	6.14	1.1	2
29					7.3			6.3	6.80	0.4	2
30					8.1			6.9	7.49	0.4	2

TABLE A4.9

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.4)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
2	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
4	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
6	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
8	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
10	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
12	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
14	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
16	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
18	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
20	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
22	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
24	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
26	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
28	0	0	0	0	0	0	0	0	0.00	0	8
	0	0	0	0	0	0	0	0	0.00	0	8
30	0	0	0	0	0	0	0	0	0.00	0	8

TABLE A4.10

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.5)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
3	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
7	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
8	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
9	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
10	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
11	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
12	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
13	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
14	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
15	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
16	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
17	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
18	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
19	0.0	0.0	0.0	0.0	5.3	0.0	0.88	0.8	6
20	0.0	0.0	0.0	0.0	5.3	0.0	0.88	0.8	6
21	0.0	0.0	0.0	0.0	5.3	0.0	0.88	0.8	6
22	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
23	0.0	0.0	5.3	5.3	0.0	5.3	2.65	1.1	6
24	0.0	0.0	5.3	5.3	0.0	5.3	2.65	1.1	6
25	0.0	0.0	5.3	5.3	0.0	5.3	2.65	1.1	6
26	0.0	0.0	5.3	5.3	0.0	5.3	2.65	1.1	6
27	5.3	5.3	5.3	5.3	5.3	5.3	5.30	0.0	6
28	0.0	0.0	6.3	0.0	5.6	5.3	2.87	1.2	6
29	5.3	5.6	6.9	5.3	5.6	6.9	5.93	0.3	6
30	5.3	5.3	6.6	5.3	5.6	5.3	5.57	0.2	6

TABLE A4.11

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^4 organisms (Experiment 4.5)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
8	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
10	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
12	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
14	4.6	8.6	6.6	5.3	0.0	4.6	4.95	1.1	6
	4.6	8.4	6.6	6.6	0.0	4.6	5.13	1.1	6
16	5.3	5.3	5.3	5.3	0.0	4.6	4.30	0.8	6
	5.3	7.5	7.2	6.6	5.3	5.3	6.20	0.4	6
18	5.3	7.9	7.2	6.6	5.3	5.3	6.27	0.4	6
	5.3	8.3	5.3	5.3	5.3	5.3	5.80	0.5	6
20	5.3	8.6	5.3	5.3	5.3	5.3	5.85	0.5	6
	0.0	8.2	5.3	7.1	6.9	5.9	5.56	1.1	6
22	0.0	7.5	5.3	6.9	7.5	7.2	5.73	1.1	6
	6.6	7.7	6.6	7.2	7.7	5.6	6.89	0.3	6
24	6.9	8.2	5.3	7.1	5.9	6.6	6.67	0.4	6
	6.6	8.2	7.2	7.6	6.4	6.6	7.11	0.3	6
26	5.3	8.2	7.5	7.9	6.6	6.6	7.02	0.4	6
	5.6	7.8	5.6	8.1	6.2	6.2	6.59	0.4	6
28	6.3	7.4	5.3	5.3	6.6	5.3	6.03	0.3	6
	6.9	7.9	6.3	5.9	7.1	7.1	6.86	0.3	6
30	6.9	6.6	6.3	6.9	6.6	6.6	6.65	0.1	6

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^5 organisms (Experiment 4.5)

Days	M1	M2	M3	M4	M5	M6	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
4	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	6
	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
8	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
10	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
12	0.0	0.0	0.0	0.0	4.6	4.6	1.53	0.9	6
	8.5	0.0	8.6	0.0	4.6	8.6	5.04	1.6	6
14	8.8	6.6	8.9	4.6	5.3	8.9	7.18	0.7	6
	9.3	6.6	9.3	5.3	5.3	9.3	7.50	0.7	6
16	9.4	5.3	8.9	5.3	5.3	8.9	7.18	0.8	6
	8.8	5.3	9.2	5.3	5.3	9.2	7.17	0.8	6
18	9.0	6.6	9.2	5.3	5.3	9.1	7.40	0.7	6
	8.9	7.5	9.6	5.3	6.9	8.9	7.86	0.6	6
20	9.0	8.1		5.3	7.5	8.8	7.75	0.6	5
	8.7	5.3		5.3	6.6	8.3	6.83	0.6	5
22	8.6	5.3		6.6	7.3	5.3	6.62	0.6	5
	7.9	7.1		5.9	6.6	5.3	6.55	0.4	5
24	6.9	8.3		5.9	7.2	5.3	6.73	0.5	5
	7.7	8.5		5.8	6.9	7.6	7.29	0.4	5
26	7.9	8.6		5.6	5.3	7.9	7.06	0.6	5
	8.4	8.6		5.9	5.6	7.5	7.21	0.6	5
28	8.0	8.7		6.3	6.6	8.1	7.54	0.4	5
	6.9	8.6		6.3	6.6	6.9	7.06	0.4	5
30	7.2	8.2		5.3	5.9	6.6	6.64	0.5	5

TABLE A4.13

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^2 organisms (Experiment 4.6)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0	0	8
1	0	0	0	0	0	0	0	0	0	0	8
2	0	0	0	0	0	0	0	0	0	0	8
3	0	0	0	0	0	0	0	0	0	0	8
4	0	0	0	0	0	0	0	0	0	0	8
5	0	0	0	0	0	0	0	0	0	0	8
6	0	0	0	0	0	0	0	0	0	0	8
7	0	0	0	0	0	0	0	0	0	0	8
8	5.9	6.6				5.4			5.97	0.28	8
9	6.2	7.5				5.4		5.6	6.18	0.41	8
10	6.9	7.8				6.9		6.9	7.13	0.19	8
11	8.1	8.1				7.5		8.1	7.95	0.13	8
12	8.1	8.1				7.8		8.1	8.03	0.06	8
13	8.7	8.1				8.1		8.7	8.40	0.15	8
14	7.2	7.2				7.8		8.7	7.73	0.31	8
15	8.1	7.2				7.8		8.1	7.80	0.18	8
16	6.3	7.8				7.8		8.1	7.50	0.35	8
17	6.9	8.1				7.5		6.0	7.13	0.39	8
18	7.2	8.1				7.5		5.0	6.95	0.59	8
19	7.5	7.5				7.5		6.9	7.35	0.13	8
20	7.8	7.2				7.8		7.5	7.58	0.12	8
21	7.5	7.5				7.5		8.1	7.65	0.13	8
22	7.8	7.5				7.5		8.1	7.73	0.12	8
23	7.5	7.5				7.8		8.1	7.73	0.12	8
24	8.4	7.8				8.1		8.7	8.25	0.17	8
25	8.4	7.8				8.1		9.0	8.33	0.22	8
26	8.4	7.5				7.8			7.90	0.22	8
27	8.4	6.9				7.8			7.70	0.36	8
28	8.1	6.9				7.8			7.60	0.29	8
29	7.8	7.2				7.8			7.60	0.16	8
30	7.8	7.8				7.8			7.80	0.00	8

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^3 organisms (Experiment 4.6)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0	0	8
1	0	0	0	0	0	0	0	0	0	0	8
2	0	0	0	0	0	0	0	0	0	0	8
3	0	0	0	0	0	0	0	0	0	0	8
4	0	0	0	0	0	0	0	0	0	0	8
5	0	0	0	0	0	0	0	0	0	0	8
6	0	0	0	5.9	0	0	5.4	0	5.65	0.2	8
7		5.4	5.6	6.6	6.6	6.6	5.9	5.4	6.01	0.2	8
8	6.6	7.1	7.1	7.5	7.2	7.2	6.6	5.4	6.84	0.2	8
9	8.1	7.5	7.2	8.1	7.5	7.5	7.5	6.9	7.54	0.1	8
10	8.1	8.4	8.1	8.4	8.1	7.8	8.4	7.5	8.10	0.1	8
11	8.7	8.7	8.1	8.7	7.8	8.1	8.1	7.8	8.25	0.1	8
12	9.0	8.7	6.9	9.4	7.5	7.8	7.2	8.1	8.08	0.3	8
13	8.7	8.7	7.5	9.2	7.8	5.0	5.4	7.8	7.51	0.5	8
14	8.7	7.8	7.8	8.7	7.8	5.4	7.5	7.5	7.65	0.3	8
15	8.4	8.4	8.4	9.0	6.9	7.2	7.8	7.8	7.99	0.2	8
16	8.7	8.4	8.7	9.0	6.3	7.8	7.8	7.8	8.06	0.3	8
17		8.4	8.1	8.7	6.9	8.4	7.8	7.8	8.01	0.2	7
18		7.8	8.1	7.5	8.1	7.8	7.5	7.8	7.80	0.1	7
19		8.1	8.1	7.5	8.1	8.1	7.5	7.5	7.84	0.1	7
20		8.1	8.1	7.5	8.1	8.1	7.5	7.2	7.80	0.1	7
21		7.5	8.1	7.8	7.8	8.1	7.8	7.5	7.80	0.1	7
22		7.8	7.8	8.1	7.8	7.8	7.8		7.85	0.0	6
23		7.2	7.8	8.7	7.8	7.8	7.8		7.85	0.2	6
24		7.2	8.1	8.1	7.8	7.8	7.8		7.80	0.1	6
25		7.5	8.4	8.4	7.8	7.5	7.8		7.90	0.2	6
26		7.2	8.4	8.4	7.8	7.8	7.8		7.90	0.2	6
27		7.2	8.4	8.7	7.8	7.8	7.8		7.95	0.2	6
28		7.5	8.1	8.4	7.5	7.5	7.8		7.80	0.1	6
29		8.1	8.1	8.4	6.9	6.9	7.8		7.70	0.2	6
30		8.1	7.8	7.8	7.2	7.2	8.1		7.70	0.2	6

TABLE A4.15

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^4 organisms (Experiment 4.6)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0	0	
1	0	0	0	0	0	0	0	0	0	0	8
2	0	0	0	0	0	0	0	0	0	0	8
3	0	0	0	0	0	0	0	0	0	0	8
4	0	0	0	0	0	0	0	0	0	0	8
5	0	0	0	0	0	0	0	0	0	0	8
6	6.6	5.9	6.6	5.4	6.6	6.6	5.9	6.6	6.28	0.2	8
7	7.5	7.2	7.5	7.5	7.5	7.5	7.2	7.2	7.39	0.1	8
8	8.1	7.8	7.8	8.1	7.8	7.8	7.5	8.1	7.88	0.1	8
9	7.8	7.8	9.0	9.4	9.4	9.0	9.2	9.0	8.83	0.2	8
10	8.4	8.1	8.1	9.0	9.2	8.4	8.7	8.1	8.50	0.1	8
11	8.4	9.0		8.7	8.7	8.1	8.4	7.2	8.36	0.2	7
12	7.2	7.8			9.0	8.1	8.1	7.2	7.90	0.3	6
13	8.1	8.4			9.0	7.8	8.1	7.8	8.20	0.2	6
14	7.8	8.1			9.0	6.6	8.1	7.5	7.85	0.3	6
15	8.1	8.1				6.9	8.4	7.8	7.86	0.2	5
16	7.8	8.1				7.8	8.7	7.8	8.04	0.2	5
17	8.1	8.7				8.4		7.8	8.25	0.2	4
18	7.8	8.1				7.8		7.5	7.80	0.1	4
19	8.1	8.1				7.5		7.8	7.88	0.1	4
20	8.1	8.1				7.2		8.1	7.88	0.2	4
21	8.1	7.8				7.2		7.5	7.65	0.2	4
22	7.8	7.5				7.5		8.1	7.73	0.1	4
23	7.5	7.5				7.5		7.8	7.58	0.1	4
24	7.8	7.5				7.5		7.5	7.58	0.1	4
25	7.8	7.8				6.9		7.8	7.58	0.2	4
26	7.5	7.5				7.2		7.8	7.50	0.1	4
27	7.5	7.5				7.5		8.1	7.65	0.1	4
28	7.8	7.8				7.2		7.8	7.65	0.1	4
29	8.1	8.1				7.2		7.8	7.80	0.2	4
30	8.1	8.1				7.2		7.5	7.73	0.2	4

TABLE A4.16

T. CONGOLENSE PARASITAEMIA AND SURVIVORS
10^5 organisms (Experiment 4.6)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0	0	8
1	0	0	0	0	0	0	0	0	0	0	8
2	0	0	0	0	0	0	0	0	0	0	8
3	0	0	0	0	0	0	0	0	0	0	8
4	6.6	6.6	6.6	5.6	6.2	5.9	5.2	6.6	6.16	0.2	8
5	7.5	7.5	7.2	7.5	7.8	8.1	7.5	7.5	7.58	0.1	8
6	7.8	7.8	7.5	7.5	7.8	8.4	7.5	8.1	7.80	0.1	8
7	8.7	8.1	8.1	8.7	8.1	9.0	8.1	8.1	8.36	0.1	8
8	9.2	7.8	8.7	7.8	8.1	9.2	9.0	7.5	8.41	0.2	8
9	9.4	9.4	9.4	9.0	8.1	9.0	8.7	9.4	9.05	0.2	8
10	9.2	7.8	7.8	7.8	7.8		8.1	8.1	8.09	0.2	7
11	8.4	7.8	7.2	7.8	8.1		8.1	7.8	7.89	0.1	7
12	8.1	8.1	7.8	7.8	7.8		7.8	8.1	7.93	0.1	7
13	8.1	7.8	8.4	7.8	8.1		8.7	8.7	8.23	0.1	7
14	7.8	8.1	7.8	7.8	7.8		7.8	8.1	7.89	0.1	7
15	7.8	8.4	8.4	8.1	8.1		8.4	8.4	8.23	0.1	7
16	8.1	8.1	8.1	8.1	8.1		8.1	8.1	8.10	0.0	7
17	7.8	8.1	8.1	8.4	8.4		8.4	8.4	8.23	0.1	7
18	7.8	8.1	8.1	8.1	8.1		8.1	8.4	8.10	0.1	7
19	8.1	8.4	8.1	8.1	8.1		8.1	8.1	8.14	0.0	7
20	8.4	9.0	8.1	8.1	7.8		8.1	7.8	8.19	0.1	7
21	7.5	7.5	7.8	7.5	7.8		7.8	7.8	7.67	0.1	7
22	7.5	7.8	8.1	8.1	7.8		8.1	7.8	7.89	0.1	7
23	8.1	7.2	8.1	8.1	7.8		6.9	6.9	7.59	0.2	7
24	8.4	5.0	8.1	6.0	8.1		7.5	7.2	7.19	0.4	7
25	8.1	5.4	8.4	7.2	7.8		8.4	7.5	7.54	0.4	7
26	8.1	7.5	8.4	7.5	7.8		7.8	7.8	7.84	0.1	7
27		7.5	8.1	8.1	7.8		7.5	7.8	7.80	0.1	6
28		7.8	8.1	7.8	7.8		7.8	7.8	7.85	0.0	6
29		8.1	7.8	7.8	8.1		8.1	8.1	8.00	0.1	6
30		8.1	7.5	7.2	7.5		8.1	8.1	7.75	0.1	6

TABLE A4.17

THE PCVs OF MICE INFECTED WITH *T. CONGOLENSE* IN EXPERIMENT 4.6

10^2 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	49	52	49	53	53	53	53	54	52.0	0.6
7	47	48	47	49	52	47	52	49	48.9	0.7
14	28	34	51	51	55	37	50	33	42.4	3.4
21	40	43	49	49	49	34	52	48	45.5	2.0
28	34	48	50	50	51	40	52		46.4	2.4

10^3 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	51	51	53	52	52	54	53	54	52.5	0.4
7	47	49	50	48	51	49	49	47	48.8	0.5
14	32	30	47	25	25	47	49	29	35.5	3.4
21		24	37	43	34	39	37	20	33.4	2.9
28		40	25	34	37	38	31		34.2	2.0

10^4 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	51	52	50	51	51	48	48	51	50.3	0.5
7	45	48	46	46	48	39	51	48	46.4	1.2
14	37	34			38	28	24	36	32.8	2.1
21	41	45				42		40	42.0	0.9
28	34	38				44		33	37.3	2.2

10^5 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	53	48	53	46	49	53	51	49	50.3	0.9
7	37	41	38	38	38	36	38	37	37.9	0.5
14	22	40	33	36	31		25	38	32.1	2.3
21	34	43	34	39	35		30	38	36.1	1.5
28		45	40	38	35		34	43	39.2	1.6

Uninfected controls

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	50	53	49	49	54	52	52	48	50.9	0.7
7	46	47	44	47	48	47	47	45	46.4	0.4
14	49	47	46	51	47	47	46	47	47.5	0.6
21	49	47	48	48	50	44	48	47	47.6	0.6
28	52	49	45	47	47	47	47	45	47.4	0.7

TABLE A4.18

THE LIVE WEIGHTS OF MICE INFECTED WITH *T. CONGOLENSE* IN EXPERIMENT 4.6

10^2 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	22	23	25	20	24	23	24	23	23.0	0.5
7	25	26	27	22	25	26	27	24	25.3	0.6
14	26	28	27	21	26	27	26	23	25.5	0.8
21	28	32	28	23	28	32	26	26	27.9	1.0
28	30	35	31	23	26	32	25		28.9	1.5

10^3 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	22	25	25	23	23	23	22	23	23.3	0.4
7	24	26	28	25	25	26	24	26	25.5	0.4
14	22	24	26	25	24	28	27	28	25.5	0.7
21		21	31	30	26	33	30	27	28.3	1.4
28		25	28	34	30	35	32		30.7	1.4

10^4 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	25	24	23	21	22	24	25	24	23.5	0.5
7	26	27	26	24	25	27	26	26	25.9	0.3
14	26	26			25	29	22	26	25.7	0.8
21	33	32				32		29	31.5	0.8
28	34	34				32		29	32.3	1.0

10^5 organisms

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	22	23	22	23	23	22	23	23	22.6	0.2
7	26	24	23	26	26	26	26	25	25.3	0.4
14	27	26	21	28	26		25	26	25.6	0.8
21	24	30	24	29	29		26	30	27.4	0.9
28		28	24	28	30		29	30	28.2	0.8

Uninfected controls

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	21	24	22	22	23	22	24	23	22.6	0.4
7	24	27	24	25	26	25	27	26	25.5	0.4
14	23	25	25	28	28	25	26	26	25.8	0.6
21	27	26	27	28	29	29	28	29	27.9	0.4
28	27	28	27	29	30	30	28	32	28.9	0.6

TABLE A4.15

ELISA VALUES FOR MICE IN EXPERIMENT 4.6

10^2 organisms (Factor=1.2497009058)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.024	-0.041				0.033		-0.042	-0.007	0.02
7	-0.041	-0.042				0.075		-0.002	-0.003	0.02
14	0.581	0.871				0.369		0.664	0.621	0.09
21	0.922	1.310				0.784		0.629	0.911	0.13
28	1.235	1.421				1.301			1.319	0.04

10^3 organisms (Factor=1.2264340825)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.036	0.007	0.012	-0.016	-0.031	-0.030	-0.006	0.010	-0.002	0.01
7	0.061	0.011	-0.041	0.062	-0.009	0.023	-0.005	-0.028	0.009	0.01
14	0.523	0.766	0.843	1.129	0.822	1.076	0.937	0.609	0.838	0.07
21		0.473	0.779	0.757	0.574	0.914	1.023	0.333	0.693	0.09
28		1.045	1.026	1.048	0.899	1.125	1.370		1.085	0.06

10^4 organisms (Factor=1.2237656904)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.006	-0.052	-0.029	0.061	-0.062	-0.071	-0.073	-0.052	-0.036	0.01
7	0.378	0.063	0.548	0.302	0.088	0.270	0.038	0.348	0.255	0.06
14	0.774	0.969			0.873	0.478	0.313	1.086	0.749	0.11
21	0.765	1.207				1.155		1.193	1.080	0.09
28	1.386	1.188				1.312		1.158	1.261	0.05

10^5 organisms (Factor=1.2093946411)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.011	-0.011	-0.046	-0.058	-0.035	-0.014	-0.011	-0.044	-0.026	0.01
7	0.548	0.302	0.158	0.454	0.406	0.355	0.227	0.226	0.334	0.04
14	0.160	0.562	0.078	0.724	0.191		0.683	0.618	0.431	0.10
21	1.092	0.746	0.173	0.983	0.715		0.997	1.088	0.828	0.11
28		1.303	1.325	1.022	1.248		0.609	1.083	1.098	0.10

Uninfected controls (Factor=1.2484206932)

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.000	-0.037	-0.030	-0.070	-0.098	-0.084	-0.092	-0.085	-0.062	0.01
7	-0.018	0.019	-0.055	-0.060	-0.074	-0.057	-0.078	-0.085	-0.051	0.01
14	-0.058	0.008	-0.037	-0.057	-0.091	-0.053	-0.104	-0.036	-0.053	0.01
21	-0.059	-0.030	-0.024	-0.024	-0.055	-0.028	-0.055	0.023	-0.031	0.01
28	-0.060	-0.040	-0.037	-0.043	-0.078	-0.043	-0.083	-0.063	-0.056	0.01

TABLE A5.1

THE PCVs OF MICE USED IN CHAPTER FIVE

60L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	54	51	50	49	50	50	52	50	50.75	0.52
7	55	50	51	50	48	53	49	48	50.50	0.81
14	53	51	49	51	50	52	51	50	50.88	0.41
21	56	48	57	50	52	54	52	50	52.38	1.03
28	53	52	50	53	53	51	51	50	51.63	0.43

125L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	55	52	52	51	51	53	53	52	52.38	0.43
7	53	49	49	52	53	51	53	56	52.00	0.77
14	53	54	50	49	45	54	52	54	51.38	1.06
21	54	50	54	51	55	50	50	57	52.63	0.90
28	52	53	51	49	55	55	53	54	52.75	0.68

250L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	53	55	51	53	54	53	49	54	52.75	0.63
7	50	49	49	52	52	51	50	52	50.63	0.43
14	50	49	49	55	53	50	47	52	50.63	0.85
21	53	54	48	55	58	52	51	54	53.13	0.98
28	53	52	54	53	56	54	51	52	53.13	0.51

500L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	54	55	51	54	53	50	53	50	52.50	0.64
7	48	53	50	47	51	52	49	52	50.25	0.70
14	48	52	47	48	47	48	47	47	48.00	0.56
21	47	52	49	52	47	50	53	52	50.25	0.79
28	52	54	52	54	49	53	55	50	52.38	0.68

Uinfected group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	53	55	52	53	55	53	52	55	53.50	0.43
7	50	49	49	52	52	51	50	52	50.63	0.43
14	53	51	51	50	52	52	48	54	51.38	0.61
21	50	54	51	54	54	50	47	54	51.75	0.88
28	52	52	52	53	55	53	53	53	52.88	0.33

TABLE A5.2

THE LIVE WEIGHTS OF MICE USED IN CHAPTER FIVE

60L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	26	24	23	26	26	29	27	26	25.88	0.60
7	25	27	25	28	28	31	26	27	27.13	0.65
14	26	26	26	29	28	32	27	28	27.75	0.68
21	28	29	26	30	28	34	27	28	28.75	0.81
28	28	28	28	30	30	35	28	28	29.38	0.81

125L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	25	23	26	29	30	26	26	27	26.50	0.73
7	26	26	26	30	29	29	28	29	27.88	0.54
14	29	26	27	32	30	30	28	29	28.88	0.62
21	29	28	19	30	32	30	28	29	28.13	1.29
28	28	27	28	33	30	30	27	30	29.13	0.67

250L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	25	26	25	29	26	25	28	27	26.38	0.50
7	25	27	28	27	25	26	26	29	26.63	0.47
14	26	29	28	32	26	28	31	29	28.63	0.71
21	26	28	25	31	26	28	33	29	28.25	0.90
28	26	31	25	33	27	27	33	29	28.88	1.04

500L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	26	28	23	26	27	26	24	27	25.88	0.54
7	28	29	24	28	28	27	27	27	27.25	0.49
14	30	31	22	28	30	28	27	25	27.63	0.98
21	32	30	23	29	30	29	28	28	28.63	0.86
28	30	29	24	29	30	28	28	28	28.25	0.63

Uinfected group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	26	25	28	29	26	25	25	27	26.38	0.50
7	25	27	28	27	25	26	26	29	26.63	0.47
14	28	27	30	28	26	28	29	30	28.25	0.46
21	28	27	30	29	27	28	31	31	28.88	0.54
28	26	27	30	28	28	26	30	29	28.00	0.53

TABLE A5.3

H. POLYGYRUS BURDENS OF MICE USED IN CHAPTER 5
[at 30 days post infection]

60L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	18	15	13	8	10	12	15	19	13.8	1.2
Female	34	15	29	23	17	26	24	22	23.8	2.0
Total	52	30	42	31	27	38	39	41	37.5	2.7
%Esta.	86.7	50.0	70.0	51.7	45.0	63.3	65.0	68.3	62.5	4.4
M:F	0.53	1.00	0.45	0.35	0.59	0.46	0.63	0.86	0.61	0.1

125L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	20	23	28	31	10	8	28	12	20.00	3.0
Female	35	42	54	38	22	25	30	15	32.63	4.1
Total	55	65	82	69	32	33	58	27	52.63	6.6
%Estab	44	52	65.6	55.2	25.6	26.4	46.4	21.6	42.10	5.3
M:F	0.57	0.55	0.52	0.82	0.45	0.32	0.93	0.80	0.62	0.1

250L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	83	52	70	74	73	58	21	82	64.13	6.8
Female	81	88	115	124	117	89	33	89	92.00	9.5
Total	164	140	185	198	190	147	54	171	156.13	15.2
%Estab	65.6	56	74	79.2	76	58.8	21.6	68.4	62.45	6.1
M:F	1.02	0.59	0.61	0.60	0.62	0.65	0.64	0.92	0.71	0.1

500L3 group

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	118	55	95	132	133	143	112	109	112.13	9.2
Female	158	135	130	243	154	175	192	140	165.88	12.4
Total	276	190	225	375	287	318	304	249	278.00	19.0
%Estab	55.2	38	45	75	57.4	63.6	60.8	49.8	55.60	3.8
M:F	0.75	0.41	0.73	0.54	0.86	0.82	0.58	0.78	0.68	0.1

TABLE 5.4

THE LENGTHS [mm] OF *H. POLYGYRUS* RECOVERED FROM MICE USED IN CHAPTER FIVE
[30 days after infection]

	60L3 group		125L3 group		250L3 group		500L3 group	
S/N	Males	Females	Males	Females	Males	Females	Males	Females
1	7.9	20.9	6.4	17.7	7.2	18.2	7.7	17.2
2	8.8	21.3	7.0	17.2	6.4	18.3	7.2	17.3
3	7.5	17.8	6.9	17.7	7.8	20.5	7.5	17.4
4	7.7	22.7	7.3	17.1	7.6	18.7	6.6	18.3
5	8.3	19.3	6.9	18.3	7.6	16.7	7.5	18.3
6	8.0	21.5	7.5	20.4	7.1	20.9	6.6	17.6
7	8.1	19.4	7.0	19.6	6.9	18.1	7.0	17.1
8	8.2	23.8	7.2	19.6	6.9	19.6	7.0	17.4
9	8.3	22.9	6.6	18.3	8.4	17.8	7.2	19.8
10	8.6	20.0	6.6	17.6	7.7	17.8	6.6	16.3
11		21.5	6.8	19.7	7.5	19.1	7.2	18.7
12		20.8	7.1	17.1	7.8	19.3	7.3	18.2
13		19.3	7.9	19.4	6.8	16.3	7.4	15.7
14		22.0	6.5	18.5	7.0	16.9	7.6	18.6
15		17.4	7.3	17.8	7.8	20.5	7.2	16.3
MEAN	8.14	20.71	6.99	18.41	7.36	18.57	7.17	17.62
SE	0.1	0.5	0.1	0.3	0.1	0.4	0.1	0.3

TABLE A5.5

THE NUMBERS OF EGGS PASSED *IN VITRO* BY *H. POLYGYRUS*
RECOVERED FROM MICE USED IN CHAPTER FIVE

[EGG/FEMALE/24hr]

GROUP	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
60L3	320	440	500	410		533	340	300	406.1	31.7
125L3	250	650	533	450	430	353	290	380	417.0	43.0
250L3	303	660	478	551	556	597	380	400	490.6	40.3
500L3	398	301	305	600	480	491	404	340	414.9	34.3

TABLE A5.6

TERMINAL BODY & SPEEN WEIGHTS [GRAM] OF MICE USED IN CHAPTER FIVE
[30 days after infection]

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
60L3 Group										
BODY	28	29	26	29	33	26	28	28	28.4	0.7
SPLEEN	0.1	0.16	0.11	0.15	0.17	0.17	0.13	0.14	0.14	0.0
%SP:BODY	0.36	0.55	0.42	0.52	0.52	0.65	0.46	0.50	0.50	0.0
125L3 Group										
BODY	28	30	29	33	32	30	26	29	29.6	0.7
SPLEEN	0.18	0.25	0.18	0.19	0.19	0.2	0.2	0.14	0.19	0.0
%SP:BODY	0.64	0.83	0.62	0.58	0.59	0.67	0.77	0.48	0.65	0.0
250L3 Group										
BODY	27	30	25	32	28	28	32	31	29.1	0.8
SPLEEN	0.19	0.16	0.14	0.17	0.2	0.24	0.25	0.21	0.20	0.0
%SP:BODY	0.70	0.53	0.56	0.53	0.71	0.86	0.78	0.68	0.67	0.0
500L3 Group										
BODY	30	28	24	27	29	27	27	29	27.6	0.6
SPLEEN	0.29	0.24	0.23	0.15	0.2	0.19	0.19	0.23	0.22	0.0
%SP:BODY	0.97	0.86	0.96	0.56	0.69	0.70	0.70	0.79	0.78	0.0
Uninfected										
BODY	25	28	31	29	28	28	30	30	28.6	0.6
SPLEEN	0.09	0.13	0.12	0.1	0.14	0.13	0.15	0.13	0.12	0.0
%SP:BODY	0.36	0.46	0.39	0.34	0.50	0.46	0.50	0.43	0.43	0.0

TABLE A5.7

ELISA VALUES (AT 450 nm) OF MICE USED IN CHAPTER FIVE

60L3 Group [Factor=4.0175332527]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.027	-0.051	-0.077	-0.018	-0.033	-0.022	-0.091	-0.010	-0.041	0.010
7	0.118	0.030	0.086	0.018	0.130	0.062	0.074	0.104	0.078	0.013
14	0.347	0.098	0.277	0.096	0.188	0.146	0.178	0.144	0.184	0.029
21	0.335	0.022	0.279	0.060	0.231	0.092	0.211	0.114	0.168	0.037
28	0.156	0.068	0.719	0.136	0.291	0.440	0.369	0.146	0.291	0.071

125L3 Group [Factor=3.1094992981]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.177	0.043	0.060	-0.007	-0.045	0.060	-0.031	0.012	0.034	0.023
7	0.259	0.133	0.051	0.124	0.070	0.140	0.116	0.096	0.124	0.021
14	0.351	0.161	0.196	0.615	-0.067	0.258	0.171	0.158	0.247	0.056
21	0.247	0.169	0.074	0.413	0.177	0.199	0.132	0.135	0.193	0.034
28	0.346	0.303	0.090	0.329	0.561	0.356	0.213	0.451	0.331	0.047

250L3 Group [Factor=4.1845088161]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.021	-0.051	0.018	-0.007	0.031	0.060	0.023	0.133	0.029	0.018
7	0.033	0.062	0.060	0.209	0.186	0.257	0.186	0.295	0.161	0.032
14	0.121	0.121	0.188	0.295	0.213	0.366	0.305	0.380	0.249	0.034
21	0.073	0.085	0.169	0.180	0.154	0.385	0.311	0.420	0.222	0.044
28	0.255	0.255	0.395	0.257	0.240	0.420	0.382	0.439	0.330	0.028

500L3 Group [Factor=3.8678696158]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.020	-0.022	0.193	0.247	-0.024	0.086	0.036	0.214	0.094	0.036
7	0.111	0.098	0.162	0.156	0.044	0.146	0.040	0.245	0.125	0.022
14	0.206	0.181	0.338	0.255	0.131	0.419	0.363	0.512	0.300	0.043
21	0.367	0.123	0.496	0.328	0.098	0.450	0.256	0.684	0.350	0.065
28	0.456	0.295	0.624	0.782	0.235	0.473	0.372	0.808	0.506	0.071

Uninfected Group [Factor=4.5859213251]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.180	0.086	0.075	0.063	0.031	-0.022	0.054	0.011	0.060	0.020
7	0.325	0.056	0.020	0.050	0.011	-0.003	0.102	0.050	0.076	0.035
14	0.334	0.056	0.008	0.150	0.050	0.001	0.171	0.187	0.120	0.037
21	0.297	0.015	0.017	0.061	-0.019	-0.001	-0.008	0.001	0.046	0.035
28	0.343	0.056	0.006	0.043	0.008	0.133	0.325	0.109	0.115	0.038

TABLE A7.1

THE *H. POLYGYRUS* BURDENS OF MICE USED IN EXPERIMENT 7.1
AND THE NUMBER EGGS PASSED *IN VITRO* IN 24 HOURS BY THE WORMS

HA50

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	114	69	71	102	119	70	111	86	92.8	7
Females	215	132	98	125	196	123	150	126	145.6	13
Total	329	201	169	227	315	193	261	212	238.4	19
Protection	87.0	44.2	53.1	37.0	12.6	45.6	27.6	41.2	33.9	5.3
Eggs	28.0	7.0	0.1	15.0	9.5	5.2	10.7	7.5	10.4	3

HA250

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	106	81	114	61	65	105	65	107	88.0	7
Females	140	94	124	69	54	135	95	162	109.1	12
Total	246	175	238	130	119	240	160	269	197.1	19
Protection	31.8	51.5	34.0	63.9	67.0	33.4	55.6	25.4	45.3	5.3
Eggs	2.4	14.9	16.8	1.5	10.1	11.7	8.8	14.9	10.1	2

HA500

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	135	72	74	22	108	108	56	30	75.6	13
Females	137	94	108	31	162	97	62	22	89.1	16
Total	272	166	182	53	270	205	118	52	164.8	28
Protection	24.5	54.0	49.5	85.3	25.1	43.1	67.3	85.6	54.3	7.9
Eggs	5.9	10.8	0.5	7.5	17.0	21.5	5.1	3.8	9.0	2

HA0

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	132	153	145	171	136	146	184	141	151.0	6
Females	184	203	239	220	154	219	218	239	209.5	10
Total	316	356	384	391	290	365	402	380	360.5	13
Estab.(%)	63.2	71.2	76.8	78.2	58.0	73.0	80.4	76.0	72.1	2.6
Eggs	74.7	62.9	129	113	84.1	125	114	45.3	93.5	10

TABLE A7.2

THE *H. POLYGYRUS* BURDENS OF MICE USED IN EXPERIMENT 7.2

HL50/1

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	143	103	0	49	0	1	95	55.9	21
Females	126	147	0	44	0	2	120	62.7	23
Total	269	250	0	93	0	3	215	119	43
Protection	19.5	25.1	100.0	72.2	100.0	99.1	35.6	64.5	12.9

HL500/1

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	0	9	0	0	15	80	0	14.9	10
Females	2	21	4	2	12	67	7	16.4	8
Total	2	30	4	2	27	147	7	31.3	18
Protection	99.4	91.0	98.8	99.4	91.9	56.0	97.9	90.6	5.5

HL50/2

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	0	21	139	60	89	53	118	68.6	18
Females	0	29	135	107	138	93	125	89.6	19
Total	0	50	274	167	227	146	243	158	36
Protection	100.0	85.0	18.0	50.0	32.0	56.3	27.2	52.7	10.7

HL500/2

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	50	0	0	1	0	0	132	26.1	18
Females	87	1	0	0	0	0	117	29.3	18
Total	137	1	0	1	0	0	249	55.4	35
Protection	59.0	99.7	100.0	99.7	100.0	100.0	25.4	83.4	10.4

HL50/3

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	100	157	0	17	38	121	38	67.3	21
Females	106	173	0	10	59	62	28	62.6	21
Total	206	330	0	27	97	183	66	130	41
Protection	38.3	1.2	100.0	91.9	71.0	45.2	80.2	61.1	12.2

HL500/3

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	0	0	0	0	0	0	0	0.0	0
Females	0	0	0	0	0	0	0	0.0	0
Total	0	0	0	0	0	0	0	0.0	0
Protection	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0

HL50/6

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	3	0	29	0	0	0	0	4.6	4
Females	6	2	20	0	3	1	1	4.7	2
Total	9	2	49	0	3	1	1	9.3	6
Protection	97.3	99.4	85.3	100.0	99.1	99.7	99.7	97.2	1.9

HL500/6

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	3	1	0	0	3	0	2	1.3	0
Females	6	15	0	0	3	0	2	3.7	2
Total	9	16	0	0	6	0	4	5.0	2
Protection	97.3	95.2	100.0	100.0	98.2	100.0	98.8	98.5	0.6

Control

	M1	M2	M3	M4	M5	M6	M7	MEAN	SE
Males	173	144	165	153	174	164	175	164	4
Females	194	186	186	142	178	153	151	170	7
Total	367	330	351	295	352	317	326	334	9
Protection	73.4	66.0	70.2	59.0	70.4	63.4	65.2	66.8	1.7

TABLE A7.3

THE LENGTHS (mm) OF *H. POLYGYRUS* RECOVERED FROM MICE IN EXPERIMENT 7.2

	IMMUNIZED		UNIMMUNIZED	
S/N	Males	Females	Males	Females
1	4.7	10.2	7.3	18.3
2	5.5	7.0	7.4	17.8
3	3.8	11.3	7.1	17.7
4	5.4	12.0	7.0	19.3
5	4.3	10.6	8.3	17.2
6	4.2	10.3	8.0	18.8
7	5.0	7.5	8.1	16.8
8	4.3	8.7	7.5	17.3
9	4.8	10.0	7.3	19.3
10	5.1	10.3	7.2	21.1
11	4.0	8.2	6.7	17.8
12	4.8	8.8	7.0	18.3
13	5.3	9.8	7.1	19.5
14	4.7	9.2	6.8	17.2
15	3.7	10.3	6.8	17.8
16	4.1	10.7	7.6	19.1
17	5.1	11.5	6.5	20.2
18	5.2	7.9	7.3	17.8
19	5.2	8.6	7.3	18.8
20	4.9	10.3	7.4	20.2
Mean	4.7	9.6	7.3	18.5
SE	0.1	0.3	0.1	0.3

TABLE A7.4

BLOOD LEUCOCYTE COUNTS (PER 100 CELLS) OF MICE USED IN EXPERIMENT 7.2

EOSINOPHILS

	M1	M2	M3	M4	M5	M6	M7	Mean	SE
HL50/1	7	6	9	4	3	1	10	5.7	1
HL500/1	3	8	6	9	7	2	0	5.0	1
HL50/2	4	9	8	7	6	17	10	8.7	1
HL500/2	18	15	18	5	4	7	22	12.7	3
HL50/3	12	12	7	15	22	11	1	11.4	2
HL500/3	16	14	10	8	11	20	8	12.4	2
HL50/6	8	9	9	12	4	11	2	7.9	1
HL500/6	15	8	10	14	21	31	18	16.7	3
HL0	9	14	4	4	7	6	2	6.6	1
Uninfected	4	9	7	9	5	4	3	5.9	1

LYMPHOCYTES

	M1	M2	M3	M4	M5	M6	M7	Mean	SE
HL50/1	52	49	42	62	50	41	45	48.7	2.5
HL500/1	74	42	65	46	58	54	53	56.0	3.8
HL50/2	43	37	55	59	45	32	44	45.0	3.3
HL500/2	48	48	41	47	27	42	33	40.9	2.8
HL50/3	37	55	44	33	36	34	57	42.3	3.5
HL500/3	35	56	55	41	20	41	35	40.4	4.4
HL50/6	40	46	45	49	37	51	38	43.7	1.9
HL500/6	31	34	56	54	33	46	42	42.3	3.6
HL0	45	37	43	40	37	43	45	41.4	1.2
Uninfected	80	64	63	58	72	66	65	66.9	2.5

NEUTROPHILS

	M1	M2	M3	M4	M5	M6	M7	Mean	SE
HL50/1	36	33	44	27	37	50	32	37.0	2.7
HL500/1	23	42	15	33	23	41	38	30.7	3.7
HL50/2	46	42	31	32	34	43	38	38.0	2.1
HL500/2	27	28	33	41	52	40	29	35.7	3.2
HL50/3	46	30	39	44	38	44	35	39.4	2.0
HL500/3	38	26	26	42	60	31	50	39.0	4.5
HL50/6	45	37	30	27	54	30	45	38.3	3.5
HL500/6	40	52	25	21	39	22	31	32.9	4.0
HL0	32	30	39	47	47	37	42	39.1	2.4
Uninfected	11	22	17	27	19	24	28	21.1	2.1

MONOCYTES

	M1	M2	M3	M4	M5	M6	M7	Mean	SE
HL50/1	5	12	5	7	10	8	13	8.6	1.1
HL500/1	0	8	14	12	12	3	9	8.3	1.8
HL50/2	7	12	6	2	15	8	8	8.3	1.5
HL500/2	7	9	8	7	17	11	16	10.7	1.5
HL50/3	5	3	10	8	4	11	7	6.9	1.1
HL500/3	11	4	9	9	9	8	7	8.1	0.8
HL50/6	7	8	16	12	5	8	15	10.1	1.5
HL500/6	14	6	9	11	7	1	9	8.1	1.4
HL0	14	19	14	9	9	14	11	12.9	1.2
Uninfected	5	5	13	6	4	6	4	6.1	1.1

TABLE A7.5

ELISA VALUES (AT 450 nm) FOR MICE USED IN EXPERIMENT 7.2

Group	M1	M2	M3	M4	M5	M6	M7	Mean	SE
HL50/1	0.188	0.229	0.195	0.212	0.125	0.136	0.129	0.173	0.01
HL500/1	0.334	0.187	0.195	0.242	0.150	0.184	0.251	0.220	0.02
HL50/2	0.227	0.188	0.109	0.161	0.193	0.193	0.172	0.177	0.01
HL500/2	0.239	0.230	0.183	0.194	0.306	0.261	0.199	0.230	0.02
HL50/3	0.326	0.226	0.159	0.472	0.221	0.238	0.206	0.264	0.04
HL500/3	0.431	0.350	0.464	0.357	0.389	0.325	0.233	0.364	0.03
HL50/6	0.334	0.580	0.313	0.456	0.402	0.423	0.455	0.423	0.03
HL500/6	0.389	0.611	0.334	0.541	0.404	0.593	0.409	0.469	0.04
HL0	0.092	0.093	0.088	0.100	0.080	0.077	0.092	0.089	0.00
Uninfected	0.048	0.041	0.063	0.058	0.048	0.054	0.049	0.051	0.00

TABLE A8.1A

T. CONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED ON DAY 0 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	6.6	6.6	6.6	6.9	6.3	6.6	6.6	6.6	6.6	0.1	8
6	7.5	7.5	7.5	7.5	6.9	7.2	7.2	7.5	7.4	0.1	8
7	7.8	7.8	7.8	8.1	7.5	7.5	7.8	7.8	7.8	0.1	8
8	7.8	9.0	8.4	8.4	7.8	8.7	8.4	8.1	8.3	0.1	8
9	8.1	8.4		8.4		7.8	8.4	7.8	8.2	0.1	6
10	7.2	8.1		9.0		7.8	8.1	7.8	8.0	0.2	6
11	8.1	8.4		8.4		8.1	7.8	8.7	8.3	0.1	6
12	8.1	8.4		8.4		8.4	8.1	8.1	8.3	0.1	6
13	7.5	7.8		7.8			7.2	5.4	7.1	0.4	5
14	7.8	7.8						6.0	7.2	0.5	3
15	8.1	7.6						7.2	7.6	0.2	3
16	8.1	7.5						7.5	7.7	0.2	3
17	7.5	7.8						8.1	7.8	0.1	3
18	7.8	7.8						8.1	7.9	0.1	3
19	7.8	7.8						8.4	8.0	0.2	3
20	7.5	8.1						8.1	7.9	0.2	3
21	7.8	8.1						8.2	8.0	0.1	3
22	7.8	8.1						8.4	8.1	0.1	3
23	7.8	8.4							8.1	0.2	2
24	7.8	7.9							7.9	0.0	2
25	7.5	7.5							7.5	0.0	2
26	7.8	8.1							8.0	0.1	2
27	7.8	7.8							7.8	0.0	2
28	8.1	7.8							8.0	0.1	2
29	8.1	7.8							8.0	0.1	2
30	8.2	7.8							8.0	0.1	2

TABLE A8.1B

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 0 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	6.0	6.6	6.0	5.0	5.0	5.0	6.0	6.6	5.8	0.2	8
6	6.6	7.2	6.6	5.0	5.4	6.6	6.6	7.2	6.4	0.3	8
7	7.5	7.5	7.5	5.0	6.0	7.8	7.8	8.0	7.1	0.4	8
8	8.1	8.1	8.4	5.0	7.2	8.1	8.4	8.1	7.7	0.4	8
9	8.1	7.5	8.1	6.9	8.1	8.4	8.1	8.4	8.0	0.2	8
10	8.4	8.0	7.8	7.5	8.3	8.1	7.5	8.1	8.0	0.1	8
11	8.1	7.5	7.5	8.1	8.4		8.1	7.8	7.9	0.1	7
12		7.5	7.8	8.1	8.4		7.8	7.8	7.9	0.1	6
13		8.1	8.1	7.2	8.4		7.8	7.8	7.9	0.2	6
14		8.0	8.0	8.1	7.8		7.8	7.8	7.9	0.0	6
15		7.8	8.1	7.8	8.4		8.1	8.1	8.1	0.1	6
16		7.5	7.9	7.2	8.2		8.1	8.1	7.8	0.1	6
17		7.8	7.8	7.5	8.4		7.8	7.8	7.9	0.1	6
18		7.5	8.1	7.5	8.4		8.4	8.4	8.1	0.2	6
19		7.8	8.1	7.8	8.1		7.9	8.1	8.0	0.1	6
20		7.5	8.1	7.9			7.8	7.8	7.8	0.1	5
21		7.5	8.0	8.1			7.5	7.8	7.8	0.1	5
22		7.2	8.0	8.1			7.5	8.0	7.8	0.2	5
23		7.2	8.1	8.1			8.0	7.8	7.8	0.2	5
24		7.2	8.0	8.1			8.0	8.4	7.9	0.2	5
25		6.6	7.8	8.1			8.2	8.1	7.8	0.3	5
26		7.5	7.9	8.1			7.8	8.2	7.9	0.1	5
27		7.2	7.9	8.1			7.8	8.2	7.8	0.2	5
28		6.9	8.1	8.0			7.9	8.3	7.8	0.2	5
29		6.6	7.9	7.8			7.8	8.2	7.7	0.2	5
30		6.0	7.8	7.5			7.5	8.2	7.4	0.3	5

TABLE A8.1C

T. CONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED ON DAY 5 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	0	0	0	0	0	0	0	0	0.0	0.0	8
11	5.0	6.6	6.0	6.3	5.4	6.6	6.6	5.4	6.0	0.2	8
12	6.0	7.2	6.9	6.9	7.2	7.5	7.5	6.0	6.9	0.2	8
13	7.2	7.9	7.8	7.8	8.1	7.8	7.9	7.5	7.8	0.1	8
14	7.8	7.8	8.4		7.8	7.8	7.8	7.8	7.9	0.1	7
15	6.3	7.7	7.8		6.9	7.5	8.1	7.5	7.4	0.2	7
16	5.4	8.1	8.1		7.8	7.9	8.1	8.1	7.6	0.3	7
17	6.9	7.8	8.1		7.2	7.5	8.0	8.1	7.7	0.2	7
18	7.2	8.1	8.1		7.2	7.5	8.0	8.3	7.8	0.2	7
19	7.8	8.4	7.8		7.8	8.1	8.3	8.4	8.1	0.1	7
20	8.1	8.4	8.1		7.5	8.4	8.4		8.2	0.1	6
21	8.1	8.4	7.8		7.8	8.4			8.1	0.1	5
22	8.1	8.3	7.9		7.8	8.1			8.0	0.1	5
23	8.3	8.3	5.4		7.9	7.9			7.6	0.5	5
24	8.0	8.1	5.0		8.3	8.1			7.5	0.6	5
25	7.9		5.4		8.1	8.2			7.4	0.6	4
26	7.8		8.3		7.2	8.1			7.9	0.2	4
27	7.2		8.1		7.2	8.1			7.7	0.2	4
28	6.9		8.1		7.5	8.2			7.7	0.3	4
29	6.9		7.8		7.5	8.1			7.6	0.2	4
30	7.2		7.2		7.8	8.1			7.6	0.2	4

TABLE A8.1D

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 5 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	5.4	0	0	5.4	0	0	0	0	1.4	0.8	8
11	6	5.4	5.4	6.6	5.4	5.4	5	5.4	5.6	0.2	8
12	7.5	6.6	7.2	7.5	7.2	7.2	5.7	6.9	7.0	0.2	8
13	8	7.8	7.8	7.8	7.5	7.5	7.2	7.5	7.6	0.1	8
14	8.1	7.8	7.8	8.1	8.1	8.1	7.8	7.5	7.9	0.1	8
15	7.8	7.8	8.1	8.3	8.1	8.1	7.8	7.5	7.9	0.1	8
16	8	7.3	8.1	8.3	7.8	7.8	8.1	7.5	7.9	0.1	8
17	8.1	8.1	8.1	7.8		8.1	8.1	7.5	8.0	0.1	7
18	8.4	8	8.4	7.8		8.1	8.7	7.5	8.1	0.1	7
19	8.1	8.1	8.1	8.1		8.1	8.1	7.8	8.1	0.0	7
20	8	8.4	8.1	7.8		7.9	8.1	7.5	8.0	0.1	7
21	7.9	8.1	8.7	6		8.4	8.3	7.8	7.9	0.3	7
22	8.1	8.1	8.4	6.9		7.6	7.9	7.5	7.8	0.2	7
23	8.2	8.2	8.4	7.5		7.8	7.8	7.8	8.0	0.1	7
24	7.9	7.8	8.1	7.5		7.8	7.5	8.1	7.8	0.1	7
25	8.1	7.5	7.6	7.6		7.8	7.8	8.1	7.8	0.1	7
26	8.1	7.8	7.5	7.6		7.2	7.8	8.1	7.7	0.1	7
27	8.1	7.5	7.5	7.5		7.5	7.8	8.1	7.7	0.1	7
28	8.1	7.2	7.8	7.5		7.8	8.1	8.1	7.8	0.1	7
29	7.8	7.2	7.9	7.5		7.8	7.8	7.8	7.7	0.1	7
30	7.5	7.2	8.1	7.7		7.8	7.5	7.5	7.6	0.1	7

TABLE A8.1E

T. CONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED ON DAY 10 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	0	0	0	0	0	0	0	0	0.0	0.0	8
11	0	0	0	0	0	0	0	0	0.0	0.0	8
12	0	0	0	0	0	0	0	0	0.0	0.0	8
13	0	0	0	0	0	0	0	0	0.0	0.0	8
14	0	0	0	0	0	0	0	0	0.0	0.0	8
15	0	0	0	0	0	0	0	0	0.0	0.0	8
16	6.0	6.0	6.0	6.9	6.0	5.0	5.0	5.4	5.8	0.2	8
17	6.9	7.5	7.2	7.2	6.9	6.0	5.0	6.9	6.7	0.3	8
18	7.5	7.8	7.8	7.8	7.2	7.5	5.4	7.8	7.4	0.3	8
19	7.8	7.5	8.1	8.3	7.2	7.5	6.3	6.0	7.3	0.3	8
20	7.5	7.9	8.3	8.4	7.5	7.5	7.5	7.2	7.7	0.1	8
21	7.2	6.9	8.1	8.1	6.9	6.9	7.2	7.5	7.4	0.2	8
22	7.9	5.4	7.8	8.3	7.2	7.5	6.9	7.2	7.3	0.3	8
23	7.5	6.9	6.0	8.7	7.2	5.4	7.2	7.2	7.0	0.3	8
24	7.2	7.2	6.6		6.0	5.0	7.3	7.5	6.7	0.3	7
25	7.2	7.8	7.2		7.2	6.9	7.5	7.7	7.4	0.1	7
26	7.5	8.1	7.6		7.2	7.2	7.5	7.8	7.6	0.1	7
27	7.8	8.1	7.8		7.5	7.5	6.9	7.5	7.6	0.1	7
28	8.0	8.1	7.9		6.9	7.8	6.0	7.5	7.5	0.3	7
29	8.1	8.1	8.1		6.9	7.8	7.2	7.5	7.7	0.2	7
30	8.3	8.1	8.2		6.9	7.8	7.5	7.8	7.8	0.2	7

TABLE A8.1F

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 10 IN EXPERIMENT 8.1

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	0	0	0	0	0	0	0	0	0.0	0.0	8
11	0	0	0	0	0	0	0	0	0.0	0.0	8
12	0	0	0	0	0	0	0	0	0.0	0.0	8
13	0	0	0	0	0	0	0	0	0.0	0.0	8
14	0	0	0	0	0	0	0	0	0.0	0.0	8
15	0	0	0	0	0	0	0	0	0.0	0.0	8
16	6.0	6.6	6.6	6.9	6.9	5.4	6.9	6.0	6.4	0.2	8
17	7.5	7.2	7.5	7.6	7.6	6.9	7.8	6.9	7.4	0.1	8
18	7.9	8.1	8.1	8.2	8.1	7.9	8.1	7.5	8.0	0.1	8
19	8.1	8.1	8.4	8.1	8.4	8.1	8.4	7.9	8.2	0.1	8
20	7.8	8.3	7.8	7.8	8.1	8.1	8.4	8.1	8.1	0.1	8
21	7.8	8.4	7.2	7.6	7.8	8.7	8.4	8.1	8.0	0.2	8
22	7.6	8.4	7.8	8.2	7.6	8.4		8.7	8.1	0.2	7
23	7.2	8.4	7.2	8.1	8.1			8.1	7.9	0.2	6
24	6.9	8.3	8.1	7.9	8.1			7.8	7.9	0.2	6
25	7.8	8.1	7.9	8.1	8.0			8.1	8.0	0.0	6
26	7.8	8.3	8.1	8.2	8.1			8.0	8.1	0.1	6
27	7.8	8.4	8.1	7.8	7.8			7.8	8.0	0.1	6
28	7.5	8.4	8.1	7.7	7.8			7.8	7.9	0.1	6
29	7.8	8.1	8.3	7.8	7.8			7.8	7.9	0.1	6
30	7.8	7.8	8.7	8.1	7.5			7.8	8.0	0.2	6

E A8.2A

ONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED AY 0 IN EXPERIMENT 8.2

M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
6.6	6.6	6.3	6.6	5.7	5.0	5.7	7.2	6.2	0.2	8
6.9	6.6	6.9	6.9	7.2	5.4	6.9	7.2	6.8	0.2	8
7.8	8.1	8.1	8.4	7.8	7.2	8.1	8.1	8.0	0.1	8
	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	0.0	7
	7.2	8.1	7.2	8.0	8.1	8.4	8.4	7.9	0.2	7
	7.5	8.1	7.8	8.1	7.2		8.1	7.8	0.1	6
	8.4	8.4	7.5	7.8	7.8			8.0	0.2	5
	8.1	7.8	5.0		8.1			7.3	0.7	4
	8.7	8.4	5.0		5.4			6.9	0.8	4
	8.1	8.4	6.0		6.0			7.1	0.6	4
	8.2	8.3	7.8		7.8			8.0	0.1	4
	8.3	8.1	7.9		7.9			8.1	0.1	4
	8.3	7.9	8.1		8.0			8.1	0.1	4
		7.8	8.4		8.1			8.1	0.1	3
		8.1	8.4		8.1			8.2	0.1	3
		8.7	8.4		8.2			8.4	0.1	3
		8.7	8.4		8.1			8.4	0.1	3
		8.4	8.4		8.1			8.3	0.1	3
		8.1	8.1		8.1			8.1	0.0	3
		8.4	8.1		8.1			8.2	0.1	3
		8.4	8.1		8.3			8.3	0.1	3
			8.1		8.3			8.2	0.1	2
			8.1					8.1	0.0	1
			8.1					8.1	0.0	1
			8.1					8.1	0.0	1

TABLE A8.2B

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 0 IN EXPERIMENT 8.2

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	5.4	5.4	5.4	5.4	5.0	5.4	5.4	5.4	5.4	0.0	8
6	5.7	6.6	5.7	5.7	5.0	6.0	6.9	6.6	6.0	0.2	8
7	6.9	6.9	6.9	6.9	5.4	6.9	6.9	7.2	6.8	0.2	8
8	7.8	7.9	7.5	7.8	7.5	7.8	7.8	8.1	7.8	0.1	8
9	8.1	8.1	7.8	8.0	7.8	8.0	7.8	8.0	8.0	0.0	8
10	8.1	8.1	7.8	8.0	7.8	8.0	7.8	8.0	8.0	0.0	8
11	7.8	7.8	7.8	8.4	7.2	8.0	8.1	8.0	7.9	0.1	8
12	7.8	8.4	6.0	7.8	8.0	7.8	7.8	7.8	7.7	0.2	8
13	7.2	8.4	5.4	8.1	8.1	8.1	7.2	8.1	7.6	0.3	8
14	7.8	7.8	7.8	7.5	7.5	8.1	7.2	8.1	7.7	0.1	8
15	8.0	7.9	7.9	8.7	7.2	7.8	7.5	8.1	7.9	0.1	8
16	8.1	8.0	8.0	8.4	7.9	7.8	7.6	8.3	8.0	0.1	8
17	8.2	8.1	8.1	8.3	8.0	7.8	7.8	8.4	8.1	0.1	8
18	8.0	7.8	8.1	8.3	8.0	7.9	7.8	8.4	8.0	0.1	8
19	8.1	6.3	8.1	8.3	8.1	8.1	7.9	8.5	7.9	0.2	8
20	8.1	7.8	8.1	8.4	8.1	8.0	7.8	8.2	8.1	0.1	8
21	8.1	7.9	8.0	8.4	8.1	8.0	7.8	8.1	8.1	0.1	8
22	8.1	8.1	7.8	8.1	8.1	8.1	8.0	8.1	8.1	0.0	8
23	8.1	8.1	7.5	8.1	8.1	8.1	7.8	8.1	8.0	0.1	8
24	8.1	8.3	7.8	8.1	8.1	8.3	7.8	8.4	8.1	0.1	8
25	8.0	8.1	7.8	8.4	8.1	8.1	7.8	8.1	8.1	0.1	8
26	7.9	8.1	7.8	8.7	8.1	7.8	7.8	8.1	8.0	0.1	8
27	8.0	8.1	7.8	8.5	8.1	7.9	7.8	8.1	8.0	0.1	8
28		8.1	7.8	8.4	7.9	8.1	7.5	8.3	8.0	0.1	7
29		8.0	7.8	8.4	7.8	8.1	7.2	8.4	8.0	0.1	7
30		8.0	7.8	8.4	7.8	8.1	7.2	8.4	8.0	0.1	7

TABLE A8.2C

T. CONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED ON DAY 5 IN EXPERIMENT 8.2

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
11	7.2	7.2	7.2	7.5	7.2	7.2	7.2	7.2	7.2	0.0	8
12	7.9	7.8	7.5	7.8	7.8	7.5	8.1	8	7.8	0.1	8
13	8.0	8.3	7.8	8.3	7.8	7.9	7.9	7.8	8.0	0.1	8
14	7.2	7.0	7.5	7.5	7.2	7.2	7.8	7.5	7.4	0.1	8
15	7.8		7.5	7.2	7.6	7.5	7.9	7.9	7.6	0.1	7
16	8.0		7.8	7.2	7.5	7.5	7.9	7.9	7.7	0.1	7
17	8.1		8.1	6.9	7.5	7.5	7.9	7.9	7.7	0.2	7
18	7.9		7.8	7.8	7.5	7.5	8.0	8.1	7.8	0.1	7
19	7.8		7.6	8.1	7.8	7.5	8.2	8.4	7.9	0.1	7
20	7.8		7.8	8.1	7.5	7.8	8.1	8.4	7.9	0.1	7
21	7.8		8.1	8.1	7.5	7.9	8.1	8.4	8.0	0.1	7
22	8.0		8.0	8.0	6.3	8.0	7.8	8.4	7.8	0.2	7
23			7.8	8.0	7.5	8.0	7.9	8.3	7.9	0.1	6
24			7.2	8.0	7.9	8.0	7.9	8.2	7.9	0.1	6
25			7.8	8.0	7.9	8.0	7.8	7.9	7.9	0.0	6
26			8.1	8.1	8.0	8.1	7.8	7.5	7.9	0.1	6
27			7.9	8.0	8.0	8.0	7.8	7.8	7.9	0.0	6
28			7.8	7.9	8.0	8.0	7.5	7.9	7.9	0.1	6
29			7.8	7.8	8.0	8.0	7.2	8.1	7.8	0.1	6
30			7.8	7.8	8.0	8.0	7.2	8.1	7.8	0.1	6

TABLE A8.2D

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 5 IN EXPERIMENT 8.2

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.0	5.4	0.0	8
11	7.2	7.2	6.9	6.9	7.2	7.2	7.2	6.0	7.0	0.1	8
12	8.0	7.8	7.9	7.9	7.9	7.8	7.8	7.8	7.9	0.0	8
13	8.1	8.0	8.1	8.1	7.9	8.1	7.8	7.9	8.0	0.0	8
14	7.9	7.5	7.8	7.9	7.5	7.9	7.2	7.5	7.7	0.1	8
15	7.8	7.8	8.4	8.1	8.1	8.3	7.5	7.8	8.0	0.1	8
16	7.8	7.8	8.3	8.1	8.0	8.1	7.5	7.5	7.9	0.1	8
17	7.9	7.8	8.1	8.1	8.0	8.1	7.8	6.0	7.7	0.2	8
18	7.9	8.1	8.1	8.1	8.1	8.1	7.8	7.5	8.0	0.1	8
19	7.9	8.4	8.1	8.1	8.4	8.4	7.9	7.8	8.1	0.1	8
20	7.9	8.4	8.4		8.3	8.1	7.9	7.8	8.1	0.1	7
21	8.1	8.7	8.7		8.2	8.1	8.1	7.8	8.2	0.1	7
22	7.8	8.1	8.7		7.9	8.4	8.1	8.0	8.1	0.1	7
23	7.9	7.8	8.4		7.5	8.4	8.3	8.1	8.1	0.1	7
24	7.9	8.1	8.4		5.7	8.4	8.4	8.2	7.9	0.3	7
25	8.0	8.3	8.1		6.9	8.1	8.3	8.1	8.0	0.2	7
26	8.1	8.1	7.9		7.2	7.8	8.1	7.9	7.9	0.1	7
27	8.0	8.1	8.0		7.5	7.8	7.9	7.9	7.9	0.1	7
28	8.0	8.1	8.0		7.8	7.8	7.8	8.0	7.9	0.0	7
29	7.9	8.1	8.0		7.9	7.8	7.8	8.0	7.9	0.0	7
30	7.9	8.1	8.0		7.9	7.8	7.8	8.0	7.9	0.0	7

E A8.2E

ONGOLENSE PARASITAEMIA IN SURVIVING MICE CONJOINTLY INFECTED AY 10 IN EXPERIMENT 8.2

M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
0	0	0	0	0	0	0	0	0.0	0.0	8
5.0	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
7.2	7.2	7.2	7.2	7.2	7.2	7.5	7.2	7.2	0.0	8
7.5	7.8	7.8	7.5	7.5	7.2	7.5	7.2	7.5	0.1	8
7.2	8.1	7.5	8.1	7.5	7.8	7.5	7.2	7.6	0.1	8
7.2	8.4	7.2	8.3	7.5	7.9	7.5	7.2	7.7	0.2	8
7.8	8.1	7.5	8.4	8.1	7.5	7.2	7.2	7.7	0.1	8
8.1	8.1	7.8	8.4	8.7	7.2	6.3	7.2	7.7	0.3	8
7.2	7.9	8.1	7.9	8.4	6.9	6.0	6.9	7.4	0.3	8
7.2	8.4	8.0	8.1	8.4	7.8	7.5	7.5	7.9	0.1	8
7.2	8.7	7.9	8.7	8.4	8.0	7.2	7.5	8.0	0.2	8
7.9	8.5	8.0	8.7	8.4	8.1	7.8	7.5	8.1	0.1	8
8.3	8.4	8.1	8.7	8.4	8.4	8.1	7.5	8.2	0.1	8
8.3	8.4	8.1	8.7	8.4	8.3	8.1	7.5	8.2	0.1	8
8.3	8.1	8.1	8.4	8.1	8.2	7.9	7.8	8.1	0.1	8
8.3		8.1	8.1	7.9	8.1	7.9	7.8	8.0	0.1	7
8.3		8.1	8.1	7.9	8.1	7.9	7.8	8.0	0.1	7

TABLE A8.2F

PARASITAEMIA IN SURVIVING MICE INFECTED WITH *T. CONGOLENSE* ALONE ON DAY 10 IN EXPERIMENT 8.2

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0	0	0	0	0	0	0	0	0.0	0.0	8
1	0	0	0	0	0	0	0	0	0.0	0.0	8
2	0	0	0	0	0	0	0	0	0.0	0.0	8
3	0	0	0	0	0	0	0	0	0.0	0.0	8
4	0	0	0	0	0	0	0	0	0.0	0.0	8
5	0	0	0	0	0	0	0	0	0.0	0.0	8
6	0	0	0	0	0	0	0	0	0.0	0.0	8
7	0	0	0	0	0	0	0	0	0.0	0.0	8
8	0	0	0	0	0	0	0	0	0.0	0.0	8
9	0	0	0	0	0	0	0	0	0.0	0.0	8
10	0	0	0	0	0	0	0	0	0.0	0.0	8
11	0	0	0	0	0	0	0	0	0.0	0.0	8
12	0	0	0	0	0	0	0	0	0.0	0.0	8
13	0	0	0	0	0	0	0	0	0.0	0.0	8
14	0	0	0	0	0	0	0	0	0.0	0.0	8
15	5.4	5.4	5.4	6.0	5.4	5.4	5.4	5.4	5.5	0.1	8
16	7.2	7.2	7.4	7.8	7.5	7.2	7.2	7.2	7.3	0.1	8
17	7.5	7.3	8.1	7.9	7.8	7.6	8.1	7.8	7.8	0.1	8
18	8.1	7.5	8.0	8.1	7.8	7.5	7.8	7.5	7.8	0.1	8
19	8.4	6.9	8.0	8.3	7.8	7.2	6.6	7.5	7.6	0.2	8
20	8.4	7.8	8.0	8.1	7.9	7.5	8.1	7.5	7.9	0.1	8
21	8.7	8.0	8.1	8.0	8.1	6.6	8.4	7.8	8.0	0.2	8
22	8.7	6.0	7.9	8.7	7.9	7.8	8.1	8.1	7.9	0.3	8
23	8.7	6.9	7.8	8.4	8.0	7.8	7.8	8.0	7.9	0.2	8
24	8.7	7.2	7.5	8.1	8.1	7.9	7.5	7.9	7.9	0.2	8
25	8.5	7.8	7.8	8.1	7.9	8.0	7.8	8.0	8.0	0.1	8
26	8.4	8.0	7.9	8.0	7.8	8.1	8.1	8.1	8.1	0.1	8
27	8.1	8.0	7.8	8.1	7.8	8.0	8.0	8.1	8.0	0.0	8
28	8.1	7.9	7.8	8.4	7.5	7.8	7.8	8.0	7.9	0.1	8
29	7.8	7.8	7.8	8.4	7.5	7.5	7.5	8.0	7.8	0.1	8
30	7.8	7.8	7.8	8.4	7.5	7.5	7.5	8.0	7.8	0.1	8

TABLE A8.3A

THE PCVs OF MICE USED IN EXPERIMENT 8.1

Conjoint (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	49	51	51	52	50	51	52	51.0	0.4
7	50	54	48	47	50	51	50	45	49.4	0.9
14	36	27		23			23	23	26.4	2.3
21	40	40						34	38.0	1.6
28	42	40							41.0	0.7

Trypanosome alone (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	53	50	51	50	51	51	51	51.1	0.3
7	48	47	49	49	46	49	47	46	47.6	0.4
14		42	38	33	28		37	33	35.2	1.8
21		42	35	40			37	29	36.6	2.0
28		40	39	35			41	25	36.0	2.6

Conjoint (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	53	52	50	50	52	51	51	51.3	0.3
7	51	50	47	51	48	52	46	49	49.3	0.7
14	43	36	41	41	37	35	37	43	39.1	1.1
21	43	28	35		40	32	28		34.3	2.3
28	35		37		38	36			36.5	0.6

Trypanosome alone (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	49	54	54	49	50	53	55	52.0	0.8
7	51	48	50	50	50	49	52	52	50.3	0.5
14	31	36	35	39	38	40	41	45	38.1	1.4
21	28	29	23	33		33	38	40	32.0	2.1
28	29	46	44	43		43	46	38	41.3	2.1

Conjoint (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	52	55	52	54	49	51	52	52.0	0.6
7	54	46	51	52	49	50	46	47	49.4	1.0
14	48	48	51	48	49	45	49	47	48.1	0.6
21	42	42	47	38	46	35	40	43	41.6	1.3
28	44	44	46		44	39	44	43	43.4	0.8

Trypanosome alone (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	55	49	50	50	51	54	51	52	51.5	0.7
7	50	45	51	47	47	49	51	50	48.8	0.7
14	51	47	48	49	50	50	50	51	49.5	0.5
21	35	35	34	34	35	35	35	42	35.6	0.9
28	40	32	40	26	41			46	37.5	2.7

Nematode alone

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	53	50	53	49	50	49	50	50.6	0.5
7	51	49	42	49	46	46	45	48	47.0	0.9
14	47	48	49	50	47	49	53	46	48.6	0.7
21	44	47	46	48	45	44	48	47	46.1	0.5
28	50	52	51	55	52	50	51	50	51.4	0.6

Uninfected control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	57	54	52	53	51	49	52	50	52.3	0.8
7	49	49	48	48	47	49	52	48	48.8	0.5
14	52	49	50	51	50	47	50	49	49.8	0.5
21	49	51	47	51	48	46	54	49	49.4	0.8
28	48	50	50	53	52	49	53	50	50.6	0.6

TABLE A8.3B

THE PCVs OF MICE USED IN EXPERIMENT 8.2

Conjoint (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	50	48	50	51	55	50	50	50.5	0.7
7	55	54	52	51	56	53	48	50	52.4	0.9
14		30	35	44		30			34.8	2.9
21			32	44		42			39.3	3.0
28				40					40.0	0.0

Trypanosome alone (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	54	49	48	53	49	50	50	50.6	0.7
7	48	51	48	48	49	49	48	48	48.6	0.4
14	32	36	46	26	38	40	30	32	35.0	2.1
21	25	46	43	28	35	41	40	31	36.1	2.5
28		41	38	26	26	30	41	32	33.4	2.3

Conjoint (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	48	50	50	48	51	50	52	48	49.6	0.5
7	52	50	47	48	55	53	55	50	51.3	1.0
14	32	28	40	36	40	36	37	34	35.4	1.3
21	30		44	42	48	42	41	30	39.6	2.4
28			42	40	38	40	44	30	39.0	1.8

Trypanosome alone (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	49	49	50	52	52	48	49	49	49.8	0.5
7	46	50	50	49	50	50	50	52	49.6	0.6
14	39	34	39	36	34	37	37	40	37.0	0.7
21	39	31	37		37	25	40	45	36.3	2.3
28	37	32	35		50	36	35	41	38.0	2.1

Conjoint (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	48	48	50	49	53	48	48	49	49.1	0.6
7	50	48	51	46	49	51	50	52	49.6	0.6
14	51	52	52	48	51	50	50	52	50.8	0.5
21	43	40	44	40	46	41	44	43	42.6	0.7
28	38	31	28	35	35	40	42	42	36.4	1.7

Trypanosome alone (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	49	48	51	50	51	54	50	50.4	0.6
7	50	50	50	48	48	46	52	50	49.3	0.6
14	47	54	50	52	49	48	51	48	49.9	0.8
21	36	46	47	30	31	38	46	32	38.3	2.4
28	34	40	40	36	40	41	41	34	38.3	1.0

Nematode alone

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	49	50	49	48	46	50	48	49.0	0.6
7	49	49	40	50	53	50	52	55	49.8	1.5
14	48	52	47	48	51	46	48	50	48.8	0.7
21	52	54	52	53	55	48	48	54	52.0	0.9
28	52	53	52	54	54	50	50	54	52.4	0.6

Uninfected control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	53	48	53	48	50	48	48	49	49.6	0.7
7	52	52	54	53	50	50	50	47	51.0	0.7
14	50	54	52	52	52	48	50	49	50.9	0.6
21	52	54	55	51	53	50	52	50	52.1	0.6
28	55	52	51	48	51	50	50	48	50.6	0.7

TABLE A8.4A

THE LIVE WEIGHTS OF MICE USED IN EXPERIMENT 8.1

Conjoint (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	23	24	22	24	25	21	26	24	23.6	0.5
7	25	25	24	25	27	21	28	26	25.1	0.7
14	28	24		26			26	21	25.0	1.1
21	31	27						25	27.7	1.4
28	34	34							34.0	0.0

Trypanosome alone (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	25	26	21	21	21	23	24	26	23.4	0.7
7	28	30	23	22	22	25	28	29	25.9	1.1
14		32	23	23	24		28	28	26.3	1.3
21		36	26	25			32	30	29.8	1.8
28		38	29	28			34	30	31.8	1.7

Conjoint (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	26	24	25	24	25	27	23	26	25.0	0.4
7	27	26	26	26	26	27	25	29	26.5	0.4
14	27	26	26	27	26	28	25	28	26.6	0.4
21	30	26	27		32	29	25		28.2	1.0
28	34		30		36	30			32.5	1.3

Trypanosome alone (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	25	25	23	24	23	26	21	24	23.9	0.5
7	26	30	25	27	26	28	21	28	26.4	0.9
14	25	30	28	29	28	29	21	30	27.5	1.0
21	26	28	29	32		30	22	32	28.4	1.2
28	28	29	31	34		31	25	32	30.0	1.0

Conjoint (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	24	26	27	24	23	23	22	25	24.3	0.6
7	26	27	30	27	26	26	23	27	26.5	0.6
14	25	25	30	28	28	26	23	29	26.8	0.8
21	28	29	33	32	29	28	25	30	29.3	0.8
28	31	31	36		30	30	25	34	31.0	1.2

Trypanosome alone (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	23	24	26	24	22	23	26	27	24.4	0.6
7	25	28	27	26	25	26	29	27	26.6	0.5
14	25	29	28	26	24	25	30	27	26.8	0.7
21	27	32	31	26	25	24	32	28	28.1	1.1
28	30	33	33	26	29			28	29.8	1.0

Nematode alone

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	23	24	24	23	26	25	26	25	24.5	0.4
7	24	28	26	25	29	27	28	27	26.8	0.6
14	26	27	27	25	30	28	29	29	27.6	0.6
21	28	28	31	26	32	29	32	30	29.5	0.7
28	28	29	30	26	31	31	33	34	30.3	0.9

Uninfected control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	25	23	25	22	24	29	25	25.0	0.7
7	30	27	24	29	24	25	32	27	27.3	1.0
14	31	29	26	29	26	25	32	28	28.3	0.8
21	30	29	25	29	26	25	30	28	27.8	0.7
28	32	29	25	31	26	26	31	30	28.8	0.9

TABLE A8.4B

THE LIVE WEIGHTS OF MICE USED IN EXPERIMENT 8.2

Conjoint (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	22	21	21	20	20	20	20	23	20.9	0.4
7	23	22	21	22	20	21	20	22	21.4	0.4
14		23	20	24		24			22.8	0.8
21			22	28		28			26.0	1.6
28				30					30.0	0.0

Trypanosome alone (day 0)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	23	21	20	21	19	21	22	21	21.0	0.4
7	24	22	22	24	22	23	24	23	23.0	0.3
14	29	26	26	27	24	24	27	25	26.0	0.6
21	29	25	28	25	27	27	28	23	26.5	0.7
28		29	29	29	28	30	30	27	28.9	0.4

Conjoint (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	21	21	20	21	21	21	20	21	20.8	0.2
7	22	22	21	22	22	22	23	22	22.0	0.2
14	24	24	24	25	24	25	24	24	24.3	0.2
21	21		27	25	27	26	27	26	25.6	0.8
28			30	28	30	28	29	26	28.5	0.6

Trypanosome alone (day 5)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	20	21	20	21	20	22	20	21	20.6	0.2
7	21	22	21	24	22	24	21	23	22.3	0.4
14	24	24	22	26	24	26	24	24	24.3	0.4
21	25	25	24		22	24	25	26	24.4	0.4
28	30	27	28		28	29	29	29	28.6	0.3

Conjoint (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	21	22	21	19	21	21	22	21	21.0	0.3
7	23	23	23	22	23	23	22	22	22.6	0.2
14	26	25	23	24	26	25	23	25	24.6	0.4
21	26	28	24	26	28	26	25	28	26.4	0.5
28	31	27	28	30	30	29	27	29	28.9	0.5

Trypanosome alone (day 10)

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	21	21	20	20	20	20	19	24	20.6	0.5
7	21	21	22	21	22	22	20	24	21.6	0.4
14	23	24	22	22	25	26	21	26	23.6	0.6
21	24	25	22	23	25	28	22	30	24.9	0.9
28	25	28	26	26	26	31	35	34	28.9	1.3

Nematode alone

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	20	21	20	21	22	23	22	22	21.4	0.4
7	21	23	21	22	23	25	23	24	22.8	0.5
14	22	26	23	25	25	27	25	25	24.8	0.5
21	22	28	24	25	25	29	25	25	25.4	0.7
28	24	28	26	28	28	30	27	27	27.3	0.6

Uninfected control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	21	22	20	21	20	23	20	18	20.6	0.5
7	22	23	22	22	22	24	22	20	22.1	0.4
14	24	26	22	24	23	26	24	22	23.9	0.5
21	24	27	24	25	24	27	25	22	24.8	0.6
28	25	28	26	27	26	27	26	23	26.0	0.5

THE PROPORTIONS (%) OF SPLEEN TO BODY WEIGHT (g) OF MICE USED IN EXPERIMENT 8.1

Conjoint (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	35	34							34.5	0.4
Spleen	3.1	3.5							3.3	0.2
%Sp/Body	8.8	10.4							9.6	0.6

Trypanosome alone (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body		37	29	27			34	29	31.2	1.7
Spleen		2.1	2.3	2.0			2.8	2.8	2.4	0.2
%Sp/Body		5.6	7.8	7.3			8.2	9.7	7.7	0.6

Conjoint (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	32		28		36	31			31.8	1.4
Spleen	1.6		1.6		3.5	2.2			2.2	0.4
%Sp/Body	4.9		5.6		9.8	7.2			6.9	0.9

Trypanosome alone (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	27	32	32	33		31	25	30	30.0	1.0
Spleen	1.8	1.5	2.9	2.6		2.0	1.8	1.9	2.1	0.2
%Sp/Body	6.6	4.8	8.9	7.8		6.3	7.2	6.4	6.9	0.4

Conjoint (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	31	30	37		32	30	26	34	31.4	1.2
Spleen	2.1	1.6	2.4		2.0	1.6	1.4	1.9	1.9	0.1
%Sp/Body	6.9	5.5	6.6		6.3	5.3	5.3	5.5	5.9	0.2

Trypanosome alone (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	30	33	34	25	27			29	29.7	1.3
Spleen	1.4	1.8	2.2	1.4	1.2			1.4	1.6	0.1
%Sp/Body	4.6	5.5	6.4	5.7	4.4			4.8	5.2	0.3

Nematode alone

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	28	29	29	27	33	31	33	32	30.3	0.8
Spleen	0.2	0.3	0.3	0.2	0.4	0.3	0.3	0.3	0.3	0.0
%Sp/Body	0.7	1.0	1.0	0.7	1.1	0.8	1.0	1.0	0.9	0.0

Uninfected

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	31	28	25	29	27	26	31	30	28.4	0.7
Spleen	0.2	0.1	0.2	0.1	0.2	0.1	0.3	0.2	0.2	0.0
%Sp/Body	0.5	0.5	0.8	0.4	0.7	0.5	0.9	0.6	0.6	0.1

THE PROPORTIONS (%) OF SPLEEN TO BODY WEIGHT (g) OF MICE USED IN EXPERIMENT 8.2

Conjoint (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body				33					33	0
Spleen				2.89					2.89	0
%Sp/Body				8.8					8.8	0

Trypanosome alone (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body		32	30		26	31	30	26	29.2	1.0
Spleen		2.2	2.1		1.5	2.7	1.3	2.0	2.0	0.2
%Sp/Body		7.0	7.0		5.9	8.5	4.4	7.5	6.7	0.5

Conjoint (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body			31	29	31	31	31	28	30.2	0.5
Spleen			2.3	2.2	2.0	1.9	2.1	1.0	1.9	0.2
%Sp/Body			7.4	7.7	6.5	6.2	6.8	3.6	6.4	0.5

Trypanosome alone (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	31	27	30		29	33	32	30	30.3	0.7
Spleen	1.5	2.4	2.5		1.9	1.5	2.9	1.7	2.0	0.2
%Sp/Body	4.9	8.8	8.3		6.5	4.7	8.9	5.6	6.8	0.6

Conjoint (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	33		29	25	29	29	27	32	29.1	1.0
Spleen	2.5		1.6	1.7	2.1	1.5	1.5	1.7	1.8	0.1
%Sp/Body	7.5		5.4	6.7	7.3	5.2	5.7	5.2	6.2	0.4

Trypanosome alone (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	29	30	28	27	29	32	26	34	29.4	0.9
Spleen	1.8	1.4	1.3	1.8	1.1	2.2	1.3	1.6	1.6	0.1
%Sp/Body	6.3	4.8	4.5	6.5	3.9	6.7	4.9	4.7	5.3	0.3

Nematode alone

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	24	30	27	27	29	31	28	28	28.0	0.7
Spleen	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0
%Sp/Body	0.4	0.6	0.7	0.6	0.6	0.6	0.7	0.5	0.6	0.0

Uninfected

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Body	25	28	25	28	26	27	27	23	26.1	0.6
Spleen	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.0
%Sp/Body	0.4	0.5	0.4	0.6	0.9	0.6	0.6	0.6	0.6	0.1

TABLE A8.7B

THE *H. POLYGYRUS* BURDENS OF MICE USED IN EXPERIMENT 8.2 (CHAPTER 8)

Conjoint (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males				126					126.0	0
Females				170					170.0	0
Total				296					296.0	0
%Estab				59.2					59.2	0

Conjoint (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males			160	140	155	170	176	160	160.2	5
Females			165	194	188	215	187	180	188.2	6
Total			325	334	343	385	363	340	348.3	8
%Estab			65	66.8	68.6	77	72.6	68	69.7	2

Conjoint (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	174		116	205	193	203	115	155	165.9	13
Females	174		153	170	210	178	102	172	165.6	11
Total	348		269	375	403	381	217	327	331.4	23
%Estab	69.6		53.8	75	80.6	76.2	43.4	65.4	66.3	5

Nematode alone

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	193	122	208	155	184	165	129	171	165.9	10
Females	182	139	261	181	161	170	186	164	180.5	12
Total	375	261	469	336	345	335	315	335	346.4	20
%Estab	75	52.2	93.8	67.2	69	67	63	67	69.3	4

TABLE A8.6A

THE LENGTHS [mm] OF *H. POLYGYRUS* RECOVERED FROM MICE
IN EXPERIMENT 8.1

	Conjoint (day 0)		Conjoint (day 5)		Conjoint (day 10)		Nematode alone	
S/N	Males	Females	Males	Females	Males	Females	Males	Females
1	6.8	15.3	7.3	17.7	6.7	13.8	7.2	18.3
2	7.3	16.8	7.0	15.4	6.8	12.3	8.2	18.3
3	6.7	16.8	7.2	16.2	7.5	15.1	6.9	17.2
4	6.6	18.7	6.8	15.8	6.8	16.7	7.7	19.3
5	6.9	17.0	8.2	16.4	7.3	15.0	8.1	19.1
6	7.2	16.8	7.0	16.3	6.0	13.5	5.8	18.5
7	7.0	17.9	8.3	15.7	6.8	13.8	6.5	16.8
8	6.1	17.5	7.5	18.0	7.1	12.3	7.5	17.9
9	6.5	18.1	6.9	16.5	7.0	13.8	7.0	17.0
10	6.0	17.5	7.1	16.7	7.1	14.9	6.3	17.3
11	6.0	17.4	7.5	18.0	6.8	15.8	6.9	17.5
12	6.3	16.0	7.0	15.7	6.5	14.3	7.5	17.8
13	6.2	15.6	6.8	15.7	6.2	13.3	6.3	17.7
14	6.5	15.3	7.9	15.3	5.8	12.9	5.8	18.2
15	7.3	17.4	7.5	16.7	5.8	11.3	7.8	19.5
Mean	6.6	16.9	7.3	16.4	6.7	13.9	7.0	18.0
SE	0.1	0.3	0.1	0.2	0.1	0.4	0.2	0.2

TABLE A8.6B

THE LENGTHS [mm] OF *H. POLYGYRUS* RECOVERED FROM MICE
IN EXPERIMENT 8.2

	Conjoint (day 0)		Conjoint (day 5)		Conjoint (day 10)		Nematode alone	
S/N	Males	Females	Males	Females	Males	Females	Males	Females
1	6.3	14.6	7.0	17.7	7.3	13.8	6.2	15.7
2	6.0	20.0	6.5	16.9	7.0	14.7	6.0	17.2
3	7.5	17.1	6.5	17.3	7.0	15.5	6.2	14.4
4	6.4	18.9	7.3	17.3	7.0	12.7	6.0	13.3
5	8.1	15.3	7.4	16.8	6.5	15.0	6.4	13.9
6	7.5	17.4	6.7	19.0	6.7	13.9	5.9	13.9
7	6.8	15.8	6.8	17.8	7.2	16.5	5.7	17.8
8	7.7	14.5	7.1	16.2	7.0	15.5	6.7	14.8
9	7.5	16.5	6.8	17.6	7.3	15.4	7.3	16.1
10	7.6	14.1	7.5	17.4	6.7	12.9	6.7	14.3
11	7.1	17.7	7.0	16.8	6.2	14.0	6.3	16.2
12	7.5	16.6	7.8	15.9	6.3	14.0	6.7	15.5
13	6.5	18.5	7.5	17.7	6.3	13.3	6.1	12.3
14	7.5	18.0	6.8	19.7	7.0	13.3	6.8	13.8
15	6.6	16.7	6.8	14.9	6.3	14.8	7.3	17.0
16	7.3	14.8	7.2	14.8	6.0	14.0	7.0	13.8
17	6.8	17.7	6.9	14.3	6.5	15.5	6.3	14.4
18	7.1	19.2	6.3	13.3	6.0	14.2	6.5	15.3
19	7.4	17.0	6.8	15.0	5.7	14.0	7.2	17.3
20	7.3	17.4	7.2	16.4	6.2	15.0	6.5	20.7
Mean	7.1	16.9	7.0	16.6	6.6	14.4	6.5	15.4
SE	0.1	0.4	0.1	0.3	0.1	0.2	0.1	0.4

TABLE A8.8A

EGGS PASSED BY *H. POLYGYRUS* RECOVERED FROM MICE IN EXPERIMENT 8.1

Group	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Conjoint (day-0)	308	241							275	24
Conjoint (day-5)	247		271		160	336			254	32
Conjoint (day-10)	363	294	367		260	160	219	573	319	47
Nematode alone	248	44	336	370	185	280	295	243	250	33

TABLE A8.8B

EGGS PASSED BY *H. POLYGYRUS* RECOVERED FROM MICE IN EXPERIMENT 8.2

Group	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Conjoint (day-0)				282						
Conjoint (day-5)			351	230	222	212	178	218	235	22
Conjoint (day-10)	355		335	323	96	310	76	246	249	38
Nematode alone	199	345	290	315	360	323	145	371	294	27

TABLE A8.7A

THE *H. POLYGYRUS* BURDENS OF MICE USED IN EXPERIMENT 8.1 (CHAPTER 8)

Conjoint (day 0)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	140	134							137.0	2
Females	185	213							199.0	10
Total	325	347							336.0	8
%Estab.	65	69.4							67.2	2

Conjoint (day 5)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	83		107		103	99			98.0	5
Females	248		119		193	177			184.3	23
Total	331		226		296	276			282.3	19
%Estab.	66.2		45.2		59.2	55.2			56.5	4

Conjoint (day 10)

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	104	151	108		148	128	126	129	127.7	6
Females	184	347	167		210	195	185	209	213.9	21
Total	288	498	275		358	323	311	338	341.6	26
%Estab.	57.6	99.6	55		71.6	64.6	62.2	67.6	68.3	5

Nematode alone

	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
Males	133	116	111	143	123	120	105	136	123.4	4
Females	172	180	147	186	170	160	158	176	168.8	4
Total	305	296	258	329	293	280	263	312	292.0	8
%Estab.	61	59.2	51.6	65.8	58.6	56	52.6	62.4	58.4	2

TABLE A8.9A

ELISA VALUES (AT 450 nm) AGAINST *H. POLYGYRUS*-DERIVED ANTIGENS
FOR MICE USED IN EXPERIMENT 8.1

Conjoint (day 0) [Factor=1.0574474857]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.024	0.010	0.029	0.019	0.005	0.018	0.059	0.015	0.022	0.008
7	0.080	0.032	0.052	0.032	0.001	0.034	0.086	0.045	0.045	0.009
14	0.072	0.012		0.003			0.007	0.033	0.025	0.011
21	0.035	0.020						0.093	0.049	0.018
28	0.047	0.033							0.040	0.005

Trypanosome alone (day 0) [Factor=1.03327631781]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.040	0.004	0.039	0.003	-0.007	-0.003	-0.023	0.002	0.007	0.007
7	0.006	-0.003	0.001	-0.003	-0.013	-0.004	-0.014	0.008	-0.003	0.003
14		0.009	0.021	-0.007	-0.019		0.009	0.028	0.007	0.007
21		0.013	-0.010	0.014			-0.021	-0.006	-0.002	0.006
28		0.053	0.011	0.027			0.020	0.039	0.030	0.007

Conjoint (day 5) [Factor=1]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.021	-0.010	0.103	0.029	0.006	0.009	-0.004	0.025	0.022	0.012
7	0.009	-0.004	0.080	0.092	0.038	0.053	-0.011	0.083	0.042	0.014
14	0.034	0.013	0.112	0.120	0.054	0.083	0.022	0.130	0.071	0.015
21	0.024	-0.012	0.049		0.013	0.041	-0.010		0.017	0.009
28	0.022		0.080		0.028	0.090			0.055	0.015

Trypanosome alone (day 5) [Factor=1.0611825679]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.052	0.062	0.015	0.012	0.000	0.010	0.014	0.053	0.027	0.008
7	0.005	0.005	0.008	0.005	-0.006	0.005	0.005	0.038	0.008	0.004
14	-0.005	-0.014	-0.001	0.000	-0.011	-0.002	0.009	0.037	0.001	0.005
21	-0.004	-0.003	-0.016	0.022		0.004	0.006	0.035	0.006	0.008
28	0.035	0.088	0.010	0.058		0.048	0.037	0.083	0.048	0.009

Conjoint (day 10) [Factor=1]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.021	-0.010	0.103	0.029	0.006	0.009	-0.004	0.025	0.022	0.012
7	0.009	-0.004	0.080	0.092	0.038	0.053	-0.011	0.083	0.042	0.014
14	0.034	0.013	0.112	0.120	0.054	0.083	0.022	0.130	0.071	0.015
21	0.024	-0.012	0.049	-0.027	0.013	0.041	-0.010	-0.018	0.008	0.009
28	0.022	-0.028	0.080		0.028	0.090	-0.020	0.002	0.025	0.016

Trypanosome alone (day 10) [Factor=1.0392555521]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.030	-0.005	-0.011	0.018	0.023	-0.015	-0.007	0.007	0.005	0.008
7	-0.004	-0.022	-0.018	0.001	0.011	-0.015	-0.013	-0.001	-0.007	0.004
14	0.005	-0.015	-0.008	0.018	0.023	0.008	0.001	0.027	0.007	0.005
21	-0.018	-0.003	0.003	0.012	-0.008	0.010	0.003	0.034	0.004	0.008
28	0.044	-0.017	-0.011	-0.021	0.032			0.075	0.017	0.015

Nematode alone [Factor=1.0326340326]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.095	0.038	-0.003	0.112	0.036	0.017	0.081	0.018	0.047	0.013
7	0.037	0.005	0.081	0.120	0.097	0.043	-0.017	0.054	0.053	0.015
14	0.026	0.064	0.133	0.236	0.184	0.071	0.039	0.093	0.106	0.024
21	0.032	0.046	0.052	0.116	0.053	0.072	0.022	0.064	0.057	0.011
28	0.066	0.125	0.079	0.245	0.080	0.176	0.102	0.246	0.140	0.024

Uninfected [Factor=1.0097249658]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.037	0.002	0.009	0.021	-0.003	0.006	-0.006	0.034	0.013	0.005
7	0.014	-0.008	-0.010	0.008	-0.020	-0.002	-0.015	0.038	0.001	0.008
14	0.011	0.017	0.013	0.017	0.003	0.004	-0.005	0.032	0.011	0.004
21	0.019	0.012	0.031	0.018	0.002	0.016	0.001	0.057	0.019	0.007
28	-0.001	-0.013	0.004	0.004	0.008	0.001	-0.010	0.032	0.003	0.005

TABLE A8.9B

ELISA VALUES (AT 450 nm) AGAINST *T. CONGOLENSE*-DERIVED ANTIGENS
FOR MICE USED IN EXPERIMENT 8.1

Conjoint (day 0) [Factor=1]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	0.000	-0.005	0.004	0.024	-0.010	-0.007	0.045	0.004	0.007
7	0.187	0.109	0.145	0.169	0.175	0.120	0.148	0.086	0.142
14	0.336	0.126		0.137			0.038	0.230	0.173
21	0.851	0.306					-0.179	0.119	0.274
28	1.172	0.714							0.943

Trypanosome alone (day 0) [Factor=1.0339366518]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	-0.017	-0.006	0.050	0.008	-0.014	-0.009	-0.036	0.003	-0.003
7	0.066	0.128	0.206	-0.008	0.009	0.144	0.086	0.166	0.097
14		0.573	0.441	0.310	0.206		0.193	0.329	0.342
21		0.998	0.380	0.809			0.182	0.865	0.603
28		1.298	1.016	1.296			0.657	1.206	1.095

Conjoint (day 5) [Factor=1.0021929825]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	0.015	-0.007	0.110	0.064	0.005	0.037	0.025	0.067	0.040
7	0.008	-0.034	0.012	0.064	-0.003	0.035	-0.002	0.029	0.013
14	0.636	0.206	0.310	0.205	0.206	0.188	0.122	0.138	0.252
21	0.865	0.171	0.327		0.305	0.325	0.074		0.311
28	1.078		0.848		0.746	0.915			0.896

Trypanosome alone (day 5) [Factor=1.0615563298]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	-0.022	-0.038	-0.045	-0.080	-0.075	-0.070	-0.076	-0.037	-0.055
7	-0.052	-0.025	-0.025	-0.071	-0.082	-0.053	-0.085	-0.034	-0.051
14	0.298	0.348	0.585	0.567	0.506	0.418	0.600	0.315	0.454
21	0.146	0.917	0.178	0.720		0.604	0.472	0.563	0.514
28	0.764	1.164	1.239	1.241		1.118	1.126	1.107	1.108

Conjoint (day 10) [Factor=1.038488851]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	0.028	0.066	0.004	0.103	0.018	-0.011	0.015	-0.010	0.027
7	-0.006	0.018	0.010	0.007	0.003	-0.002	0.000	0.018	0.008
14	0.040	0.074	0.005	0.022	0.015	0.025	0.042	0.027	0.031
21	0.250	0.731	0.733	0.415	0.948	0.210	0.428	0.958	0.584
28	0.316	0.908	1.151		1.046	0.486	0.581	1.031	0.789

Trypanosome alone (day 10) [Factor=1.0240896359]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	-0.010	-0.084	-0.080	-0.014	-0.024	-0.038	-0.045	0.004	-0.031
7	-0.036	-0.088	-0.077	-0.032	-0.048	-0.043	-0.048	-0.015	-0.048
14	-0.008	-0.049	-0.050	-0.012	-0.027	-0.021	-0.041	0.020	-0.023
21	0.602	1.013	0.382	0.721	0.619	0.562	0.638	0.464	0.625
28	0.736	0.712	0.614	0.104	0.825			1.017	0.668

Nematode alone [Factor=1.045019294]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	0.028	-0.029	-0.045	-0.028	0.036	-0.031	0.066	-0.051	-0.007
7	-0.082	-0.023	0.013	0.038	0.074	-0.003	0.001	-0.020	0.000
14	-0.070	0.100	0.115	0.090	0.159	0.053	0.072	-0.057	0.058
21	-0.099	-0.028	0.027	0.055	0.063	0.060	-0.039	-0.043	-0.001
28	-0.057	-0.031	-0.037	0.006	0.021	0.020	-0.048	0.001	-0.016

Uninfected [Factor=1.0855108888]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN
0	-0.080	-0.068	-0.090	-0.093	-0.083	-0.089	-0.099	-0.059	-0.078
7	-0.086	-0.075	-0.104	-0.111	-0.081	-0.104	-0.105	-0.060	-0.091
14	-0.079	-0.068	-0.076	-0.080	-0.063	-0.087	-0.079	-0.023	-0.069
21	-0.063	-0.070	-0.085	-0.085	-0.067	-0.079	-0.088	-0.015	-0.069
28	-0.069	-0.069	-0.081	-0.079	-0.036	-0.082	-0.076	0.012	-0.058

TABLE A9.1A

THE *T. CONGOLENSE* PARASITAEMIAS IN SURVIVING MICE USED IN CHAPTER NINE

Immunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	5.0	5.0	5.0	5.4	5.4	5.7	5.4	5.4	5.3	0.1	8
6	6.0	6.6	5.0	5.7	6.3	6.6	6.9	6.6	6.2	0.2	8
7	7.1	7.4	6.0	7.0	7.2	7.4	7.5	7.4	7.1	0.2	8
8	8.1	8.1	6.9	8.3	8.1	8.1	8.1	8.1	8.0	0.1	8
9	8.0	8.0	7.4	8.4	7.5	7.7	8.0	8.3	7.9	0.1	8
10	7.8	7.8	7.8	8.4	6.9	7.2	7.8	8.4	7.8	0.2	8
11	7.9	8.0	7.4	8.1	7.4	7.6	7.4	8.2	7.7	0.1	8
12	7.9	8.1	6.9	7.8	7.8	7.9	6.9	8.0	7.7	0.2	8
13	7.9	8.0	7.1	8.1	7.8	7.7	7.5	6.7	7.6	0.2	8
14	7.8	7.9	7.2	8.3	7.8	7.5	8.0	5.4	7.5	0.3	8
15	8.0	8.0	7.7		8.0	7.7	8.0	6.7	7.7	0.2	7
16	8.1	8.1	8.1		8.1	7.8	8.0	7.9	8.0	0.0	7
17	8.1	8.1	8.2		8.1	7.9	8.1	8.0	8.1	0.0	7
18	8.0	8.1	8.3		8.1	7.9	8.1	8.1	8.1	0.0	7
19	8.0		8.3		8.1	7.9	7.8	8.2	8.0	0.1	6
20	8.0		8.3		8.1	7.8	7.5	8.3	8.0	0.1	6
21	8.2		8.1		8.1	7.9	7.7	8.3	8.0	0.1	6
22	8.3		7.9		8.1	7.9	7.9	8.2	8.1	0.1	6
23			7.9		8.0	8.0	7.9	8.4	8.0	0.1	5
24			7.8		7.9	8.0	7.9	8.5	8.0	0.1	5
25			7.7		8.0	8.0	7.7	8.6	8.0	0.2	5
26			7.5		8.0	8.0	7.5	8.7	7.9	0.2	5
27			7.7		8.0	7.6	7.4		7.7	0.1	4
28			7.8		8.0	7.2	7.2		7.6	0.2	4
29			7.8		8.0	7.2	7.2		7.6	0.2	4
30			7.8		8.0	7.2	7.2		7.6	0.2	4

TABLE A9.1B

THE *T. CONGOLENSE* PARASITAEMIAS IN SURVIVING MICE USED IN CHAPTER NINE

Immunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	0.0	5.4	5.4	5.4	0.0	6.9	5.4	5.4	4.2	0.9	8
6	0.0	6.0	5.7	6.9	6.0	7.2	6.9	5.7	5.6	0.8	8
7	3.6	7.1	6.6	7.6	7.1	7.7	7.5	6.9	6.7	0.4	8
8	7.2	8.1	7.5	8.3	8.1	8.1	8.1	8.1	7.9	0.1	8
9	7.7	8.3	7.8	8.5	8.0	8.0	8.2	8.3	8.1	0.1	8
10	8.2	8.4	8.0	8.7	7.8	7.8	8.3	8.4	8.2	0.1	8
11	8.2	8.2	8.0	8.5	7.7	7.7	8.2	8.4	8.1	0.1	8
12	8.1	7.9	7.9	8.3	7.5	7.5	8.1	8.3	8.0	0.1	8
13	8.1	8.0	7.7	8.2	7.8	7.8	8.1	8.5	8.0	0.1	8
14	8.1	8.0	7.5	8.1	8.1	8.0	8.1	8.7	8.1	0.1	8
15	8.3	8.1	7.7	8.4	8.1	7.9	8.1	8.8	8.1	0.1	8
16	8.4	8.1	7.8	8.6	8.0	7.8	8.1	8.8	8.2	0.1	8
17	8.4	8.1	7.8	8.1	8.0	7.8	8.2	8.6	8.1	0.1	8
18	8.3	8.1	7.8	7.5	7.9	7.8	8.2	8.4	8.0	0.1	8
19	8.3	7.5	8.0	8.0	7.9	7.8	8.1	8.3	8.0	0.1	8
20	8.2	6.9	8.1	8.5	7.8	7.8	7.9	8.1	7.9	0.2	8
21	8.2	7.4	8.1	8.5	7.7	7.9	7.9	8.1	7.9	0.1	8
22	8.1	7.8	8.1	8.4	7.5	7.9	7.8	8.0	8.0	0.1	8
23	8.2	8.0	8.1	8.3	7.7	7.9	7.8		8.0	0.1	7
24	8.2	8.1	8.1	8.1	7.8	7.8	7.8		8.0	0.1	7
25	8.5	8.0	8.0	8.0	7.8	7.8	7.8		8.0	0.1	7
26	8.7	7.9	7.8	7.8	7.8	7.8	7.8		7.9	0.1	7
27	8.4	8.0	7.7	8.1	7.8	7.7	7.4		7.8	0.1	7
28	8.0	8.0	7.5	8.3	7.8	7.5	6.9		7.7	0.2	7
29	8.0	8.0	7.5	8.3	7.8	7.5	6.9		7.7	0.2	7
30	8.0	8.0	7.5	8.3	7.8	7.5	6.9		7.7	0.2	7

TABLE A9.1C

THE *T. CONGOLENSE* PARASITAEMIAS IN SURVIVING MICE USED IN CHAPTER NINE

Unimmunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	0.0	0.0	5.4	0.0	5.4	0.0	0.0	0.0	1.4	0.8	8
6	6.0	6.9	6.0	6.0	6.0	6.0	6.0	5.7	6.1	0.1	8
7	7.2	7.5	7.2	7.1	7.0	7.1	6.8	6.9	7.1	0.1	8
8	8.3	8.1	8.3	8.1	7.9	8.1	7.5	8.0	8.0	0.1	8
9	8.4	8.1	8.4	8.0	8.1	8.0	8.1	8.2	8.1	0.1	8
10	8.4	8.1	8.4	7.8		7.8	8.7	8.4	8.2	0.1	7
11		8.0	8.2	7.7		7.7	8.4	8.3	8.0	0.1	6
12		7.9	7.9	7.5		7.5	8.1	8.1	7.8	0.1	6
13		8.0	7.9	8.0		7.8	8.3	8.0	8.0	0.1	6
14		8.0	7.9	8.4		8.1	8.4	7.8	8.1	0.1	6
15		8.2	8.2	8.3		8.0	8.4	7.9	8.1	0.1	6
16		8.4	8.4	8.1		7.8	8.4		8.2	0.1	5
17		8.4	8.4	8.1		7.9	8.3		8.2	0.1	5
18		8.3	8.4	8.1		7.9	8.2		8.2	0.1	5
19		8.2	8.4	8.3		7.9	8.1		8.1	0.1	5
20		8.0	8.4	8.4		7.8	7.9		8.1	0.1	5
21		8.0	7.7	8.2		8.1	8.0		8.0	0.1	5
22		8.0	6.9	8.0		8.3	8.0		7.8	0.2	5
23		8.0	7.7	8.1		8.1	8.1		8.0	0.1	5
24		7.9	8.4	8.1		7.9	8.2		8.1	0.1	5
25		8.0	8.3	8.1		7.7	8.2		8.0	0.1	5
26		8.1	8.1	8.0		7.5	8.1		8.0	0.1	5
27		7.8	8.0	8.0		7.5	7.8		7.8	0.1	5
28		7.5	7.9	7.9		7.5	7.5		7.7	0.1	5
29		7.8	7.7	8.1		7.5	7.2		7.7	0.1	5
30		8.0	7.5	8.3		7.5	6.9		7.6	0.2	5

TABLE A9.1D

THE *T. CONGOLENSE* PARASITAEMIAS IN SURVIVING MICE USED IN CHAPTER NINE

Unimmunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	0.0	5.4	0.0	0.0	0.0	5.4	5.4	0.0	2.0	0.9	8
6	6.0	6.0	5.4	5.4	5.4	6.9	5.4	6.0	5.8	0.2	8
7	7.1	6.9	6.6	6.7	6.7	7.5	6.8	7.2	6.9	0.1	8
8	8.1	7.8	7.8	7.9	7.9	8.1	8.1	8.3	8.0	0.1	8
9	8.4	8.1	8.1	8.3	8.0	8.1	8.4	8.4	8.2	0.1	8
10	8.6	8.3	8.3	8.7	8.1		8.7	8.4	8.4	0.1	7
11	8.7	8.2	8.2	8.7	8.1		8.5	7.7	8.3	0.1	7
12	8.7	8.0	8.1		8.1		8.3	6.9	8.0	0.2	6
13	8.9	8.1	8.0		8.1		8.5	7.4	8.1	0.2	6
14	9.0	8.1	7.8		8.1		8.7	7.8	8.3	0.2	6
15	8.7	7.8	7.8		8.2		8.7	8.3	8.3	0.2	6
16	8.4	7.5			8.3			8.6	8.3	0.2	4
17	8.4	7.2			8.3			8.5	8.1	0.3	4
18	8.4	6.9						8.1	7.8	0.4	3
19	8.4	7.2						8.1	7.9	0.3	3
20	8.4	7.5						8.1	8.0	0.2	3
21	8.3	7.5						8.3	8.0	0.2	3
22		7.5						8.4	8.0	0.3	2
23		7.5						8.4	8.0	0.3	2
24		7.5						8.4	8.0	0.3	2
25		7.8						8.3	8.1	0.2	2
26		8.1						8.2	8.2	0.0	2
27		8.0						8.2	8.1	0.1	2
28		7.8						8.1	8.0	0.1	2
29		7.9						8.1	8.0	0.1	2
30		8.0						8.1	8.1	0.0	2

TABLE 9.2

THE PCVs (%) OF MICE USED IN CHAPTER NINE

Immunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	52	55	55	57	54	54	52	54	54.1	0.5
7	50	50	47	46	49	48	49	45	48.0	0.6
14	31	25	48	31	34	32	33	40	34.3	2.3
21	34		38		39	46	44	42	40.5	1.6
28			46		30	39	42		39.3	2.9

Immunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	54	54	51	50	54	55	53	53	53.0	0.6
7	49	48	44	46	45	51	48	48	47.4	0.7
14	38	47	47	36	45	47	46	50	44.5	1.6
21	43	52	42	42	49	52	49	54	47.9	1.6
28	53	53	49	34	50	53	53	53	49.8	2.2

Immunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	56	56	56	56	56	52	53	55	55.0	0.5
7	51	50	51	44	51	44	48	49	48.5	1.0
14	40	34	36	43	50	40	29	45	39.6	2.2
21	32	47	44	34	48	40	43	28	39.5	2.4
28	37	37	41	39	43	38	44		39.9	1.0

Immunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	54	52	57	55	50	50	50	50	52.3	0.9
7	51	48	52	50	49	55	49	50	50.5	0.7
14	51	52	53	49	48	54	49	51	50.9	0.7
21	50	50	50	52	49	53	48	51	50.4	0.5
28	47	51	53	46	46	51	48	51	49.1	0.9

Unimmunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	48	48	46	50	51	53	52	49.8	0.8
7	51	44	46	51	44	48	55	50	48.6	1.3
14		31	26	43		42	34	22	33.0	3.1
21		39	29	40		47	34		37.8	5.0
28		40	36	40		45	37		39.6	5.0

Unimmunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	48	52	55	53	54	52	50	51.9	0.7
7	50	49	48	52	50	48	48	49	49.3	0.5
14	51	52	47	50	44	46	47	50	48.4	0.9
21	52	49	54	50	53	51	54	53	52.0	0.6
28	53	50	50	50	53	52	54	53	51.9	0.5

Unimmunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	54	52	51	50	50	48	50	50	50.6	0.6
7	48	44	50	46	50	48	48	46	47.5	0.7
14	28	25	26		24		32	47	30.3	3.2
21		38						42	40.0	1.2
28		36						28	32.0	4.0

Unimmunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	48	48	48	51	53	52	50	52	50.3	0.7
7	52	48	51	49	50	48	48	48	49.3	0.5
14	51	50	52	50	50	52	52	52	51.1	0.3
21	49	48	52	50	48	51	49	46	49.1	0.6
28	45	48	48	48	50	51	50	49	48.6	0.6

TABLE 9.3

THE LIVE WEIGHTS (g) OF MICE USED IN CHAPTER NINE

Immunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	25	29	29	27	27	27	27	27	27.3	0.4
7	26	28	31	31	28	29	29	30	29.0	0.6
14	26	28	34	33	32	30	29	32	30.5	0.9
21	28		35		34	33	32	33	32.5	0.9
28			37		32	38	36		35.8	1.1

Immunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	26	27	27	26	29	27	29	29	27.5	0.4
7	27	27	28	26	31	28	31	29	28.4	0.6
14	28	30	29	27	32	29	31	30	29.5	0.5
21	29	27	28	27	30	29	31	30	28.9	0.5
28	28	27	28	26	31	30	32	28	28.8	0.7

Immunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	30	28	28	27	27	25	26	27	27.3	0.5
7	30	29	29	27	31	28	27	29	28.8	0.5
14	36	32	30	32	34	30	28	33	31.9	0.8
21	38	31	32	33	33	31	31	32	32.6	0.8
28	41	34	35	36	37	37	35		36.4	0.8

Immunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	23	27	29	26	30	26	24	31	27.0	0.9
7	25	31	33	29	33	27	26	32	29.5	1.1
14	27	31	33	30	34	27	26	32	30.0	1.0
21	25	31	33	28	35	28	27	32	29.9	1.1
28	26	31	33	27	35	28	26	32	29.8	1.1

Unimmunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	28	29	25	26	25	27	26	26	26.5	0.5
7	26	32	26	28	26	27	26	26	27.1	0.7
14		25	32	32		30	30	24	28.8	1.3
21		34	28	34		33	30		31.8	5.0
28		40	36	36		36	37		37.0	5.0

Unimmunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	26	26	25	29	28	28	25	27	26.8	0.5
7	30	28	28	34	31	29	28	32	30.0	0.7
14	31	27	27	32	31	28	28	31	29.4	0.7
21	31	27	30	33	30	30	29	32	30.3	0.6
28	30	28	33	33	30	29	29	35	30.9	0.8

Unimmunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	24	28	28	25	25	29	27	26	26.5	0.6
7	25	31	30	26	27	32	29	28	28.5	0.8
14	26	29	28		28		33	30	29.0	0.9
21	27	26						32	28.3	1.5
28		38							38.0	0.0

Unimmunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	26	28	25	27	25	26	27	26.4	0.4
7	27	29	28	28	29	27	27	29	28.0	0.3
14	29	28	29	27	27	30	29	29	28.5	0.4
21	27	31	28	27	29	29	29	29	28.6	0.4
28	30	33	29	28	28	28	28	30	29.3	0.6

TABLE A9.4

PROPORTIONS (%) OF SPLEEN TO EVISCERATED CARCASS WEIGHT (g) OF MICE USED IN CHAPTER NINE

Immunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass			21		21	17	22		20.3	1.0
Spleen			3.1		2.6	2.6	2.9		2.8	0.1
%Sp/Car.			14.6		12.3	15.1	13.2		13.8	0.6

Immunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass	20	19	20	16	21	20	21	19	19.5	0.5
Spleen	0.2	0.2	0.2	0.5	0.2	0.2	0.2	0.2	0.2	0.0
%Sp/Car.	1.1	0.9	0.8	3.2	0.9	1.0	1.1	1.3	1.3	0.3

Immunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass	22	19	20	21	23	21	20		20.9	0.5
Spleen	3.7	2.7	2.7	3.1	2.5	2.2	2.9		2.9	0.2
%Sp/Car.	16.9	14.4	13.7	14.8	11.0	10.6	14.6		13.7	0.8

Immunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass	19	23	24	20	23	21	19	21	21.3	0.6
Spleen	0.3	0.2	0.2	0.1	0.3	0.2	0.1	0.1	0.2	0.0
%Sp/Car.	1.7	0.7	0.8	0.7	1.1	0.7	0.6	0.6	0.9	0.1

Unimmunized/Conjoint

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass		22	19	20		20	18		19.8	0.6
Spleen		3.2	2.9	2.7		2.9	2.9		2.9	0.1
%Sp/Car.		14.5	15.0	13.5		14.4	16.0		14.7	0.4

Unimmunized/Nematode

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass	21	19	21	21	21	20	21	24	21.0	0.5
Spleen	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.3	0.2	0.0
%Sp/Car.	0.7	1.1	1.0	0.8	0.8	0.8	0.6	1.0	0.9	0.1

Unimmunized/Trypanosome

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass		17							17.0	0.0
Spleen		2.8							2.8	0.0
%Sp/Car.		16.4							16.4	0.0

Unimmunized/Control

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Carcass	23	24	21	20	22	21	21	23	21.9	0.4
Spleen	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
%Sp/Car.	0.7	0.7	0.7	0.7	0.6	0.7	0.5	0.4	0.6	0.0

TABLE A9.5

H. POLYGYRUS BURDEN IN SURVIVING MICE USED IN CHAPTER NINE

Immunized/Conjoint

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males			114		95	130	114		113.3	6.2
Females			157		105	143	156		140.3	10.5
Total			271		200	273	270		253.5	15.5
%Estab.			54.2		40	54.6	54		50.7	3.1

Immunized/Nematode

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males	88	92	9	70	11	45	21	56	49.0	8.2
Females	96	100	9	91	21	69	28	41	56.9	10.1
Total	184	192	18	161	32	114	49	97	105.9	17.7
%Estab	36.8	38.4	3.6	32.2	6.4	22.8	9.8	19.4	21.2	3.5

Unimmunized/Conjoint

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males		133	118	160		119	96		125.2	9.4
Females		149	159	172		151	93		144.8	12.1
Total		282	277	332		270	189		270.0	20.6
%Estab		56.4	55.4	66.4		54	37.8		54.0	4.1

Unimmunized/Nematode

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males	96	123	120	110	128	164	122	93	119.5	7.3
Females	124	144	160	117	144	212	128	116	143.1	10.5
Total	220	267	280	227	272	376	250	209	262.6	17.4
%Estab	44	53.4	56	45.4	54.4	75.2	50	41.8	52.5	3.5

TABLE A9.6

NUMBERS OF EGGS PASSED *IN VITRO* BY WORMS RECOVERED FROM MICE IN CHAPTER NINE

Group	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Immunized/Conjoint			164		162	176	190		173.1	5.5
Immunized/Nematode	198	270	44	251	29	194	86	210	160.1	31.0
Unimmunized/Conjoint		203	258	157		237	219		214.8	15.3
Unimmunized/Nematode	434	214	243	299	233	312	200	243	272.3	25.1

TABLE A9.7

LENGTHS (mm) OF *H. POLYGYRUS* RECOVERED FROM MICE IN CHAPTER NINE

	Immunized/ Conjoint		Immunized/ Nematode		Unimmunized/ Conjoint		Unimmunized/ Nematode	
S/N	Males	Females	Males	Females	Males	Females	Males	Females
1	6.1	15.5	7.3	12.5	5.8	16.2	7.3	15.7
2	6.8	13.7	7.1	15.7	5.7	15.4	7.2	17.8
3	6.3	15.9	7.7	11.8	7.3	18.3	6.2	16.6
4	6.8	16.5	7.0	12.8	7.3	19.1	7.2	16.4
5	6.2	15.8	5.5	14.8	7.7	19.5	6.9	15.2
6	6.6	14.7	7.2	15.5	6.9	18.7	6.8	19.0
7	6.3	15.9	6.2	14.8	6.4	17.2	6.9	14.0
8	7.1	16.5	5.7	14.2	6.1	16.8	7.1	19.3
9	6.9	16.9	6.7	15.3	6.8	17.6	6.9	13.8
10	6.3	15.3	5.3	12.7	7.3	16.1	6.8	17.3
11	7.7	17.1	7.1	11.8	7.2	17.3	7.0	18.5
12	6.5	18.3	6.3	11.7	6.3	15.5	6.7	18.0
13	6.0	14.2	6.7	17.2	7.2	18.2	6.8	15.5
14	6.8	17.7	6.5	13.8	6.3	17.8	6.8	18.0
15	7.6	16.5	6.3	14.2	6.4	18.1	6.9	15.7
16	7.5	16.3	7.1	12.1	7.1	18.4	6.7	15.5
17	6.8	18.7	5.8	11.3	5.7	17.3	6.3	17.7
18	7.7	17.1	6.2	16.3	6.4	19.0	7.3	14.7
19	7.8	16.3	7.9	11.7	6.8	17.7	6.3	15.7
20	7.2	16.4	5.3	15.7	6.3	17.1	6.6	15.6
Mean	6.85	16.25	6.54	13.79	6.64	17.55	6.83	16.49
SE	0.13	0.27	0.17	0.39	0.13	0.25	0.07	0.35

TABLE A9.9D

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS) OF MICE IN 'IMMUNIZED/CONTROL' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	73	74	80	89	70	43	76	83	73.5	4.5
Neutrophils	15	21	10	9	20	48	15	9	18.4	4.2
Eosinophils	4	2	5	0	8	2	6	1	3.5	0.9
Monocytes	8	3	5	2	2	7	3	7	4.6	0.8
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	80	81	73	85	83	83	75	77	79.6	1.4
Neutrophils	9	10	18	11	14	15	17	13	13.4	1.1
Eosinophils	0	1	0	1	0	0	0	0	0.3	0.2
Monocytes	11	6	9	3	3	2	7	10	6.4	1.1
Basophils	0	2	0	0	0	0	1	0	0.4	0.2

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	70	68	72	83	75	74	80	82	75.5	1.9
Neutrophils	20	17	18	8	13	18	16	13	15.4	1.3
Eosinophils	1	4	4	1	3	3	4	3	2.9	0.4
Monocytes	9	11	6	8	9	5	0	2	6.3	1.2
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	82	81	85	87	86	91	86	83	85.1	1.1
Neutrophils	13	17	7	7	6	2	4	7	7.9	1.6
Eosinophils	5	2	4	1	1	4	3	3	2.9	0.5
Monocytes	0	0	4	5	7	3	7	7	4.1	1.0
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	64	63	51	49	64	60	49	73	59.1	2.9
Neutrophils	28	26	42	42	22	30	37	22	31.1	2.7
Eosinophils	2	0	3	2	3	6	5	3	3.0	0.6
Monocytes	6	11	4	7	11	4	9	2	6.8	1.1
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

TABLE A9.9E

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS) OF MICE IN 'UNIMMUNIZED/CONJOINT' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	85	75	68	88	84	80	91	94	83.1	2.8
Neutrophils	4	15	24	9	14	16	6	6	11.8	2.2
Eosinophils	5	5	6	3	2	3	2	0	3.3	0.7
Monocytes	6	5	1	0	0	1	1	0	1.8	0.8
Basophils	0	0	1	0	0	0	0	0	0.1	0.1

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	73	54	64	68	71	70	79	58	67.1	2.7
Neutrophils	22	36	22	26	19	26	10	41	25.3	3.2
Eosinophils	0	1	1	1	0	1	0	0	0.5	0.2
Monocytes	5	9	13	5	10	3	11	1	7.1	1.4
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes		32	55	26		27	50	33	37.2	4.6
Neutrophils		44	27	60		48	31	53	43.8	4.7
Eosinophils		6	5	2		7	4	3	4.5	0.7
Monocytes		18	13	12		18	15	11	14.5	1.1
Basophils		0	0	0		0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes		56	44	56		29	44		45.8	4.5
Neutrophils		15	34	13		38	18		23.6	4.6
Eosinophils		2	1	0		3	1		1.4	0.5
Monocytes		27	21	31		30	37		29.2	2.3
Basophils		0	0	0		0	0		0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes		18	36	16		19	24		22.6	3.2
Neutrophils		45	20	28		41	30		32.8	4.1
Eosinophils		4	2	0		4	5		3.0	0.8
Monocytes		33	42	56		36	41		41.6	3.5
Basophils		0	0	0		0	0		0.0	0.0

TABLE A9.9F

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS) OF MICE IN 'UNIMMUNIZED/NEMATODE' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	83	88	78	86	87	79	88	89	84.8	1.4
Neutrophils	13	6	17	9	7	19	4	8	10.4	1.8
Eosinophils	3	2	1	5	3	2	7	3	3.3	0.6
Monocytes	1	4	4	0	3	0	1	0	1.6	0.6
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	52	48	50	50	61	43	59	49	51.5	1.9
Neutrophils	42	38	39	39	30	48	33	43	39.0	1.9
Eosinophils	0	1	0	0	0	1	0	0	0.3	0.2
Monocytes	5	12	11	11	9	8	8	8	9.0	0.7
Basophils	1	1	0	0	0	0	0	0	0.3	0.2

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	50	52	64	43	40	40	47	51	48.4	2.6
Neutrophils	22	22	15	23	21	38	16	10	20.9	2.7
Eosinophils	15	17	12	23	30	18	30	28	21.6	2.4
Monocytes	13	9	9	11	9	4	7	11	9.1	0.9
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	64	69	79	66	65	64	75	62	68.0	2.0
Neutrophils	21	18	17	20	21	17	14	24	19.0	1.0
Eosinophils	5	4	1	4	5	6	6	5	4.5	0.5
Monocytes	10	9	3	10	9	13	5	9	8.5	1.0
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	43	39	41	45	50	39	33	48	42.3	1.8
Neutrophils	39	27	32	23	21	42	49	40	34.1	3.3
Eosinophils	8	18	9	16	16	8	7	5	10.9	1.6
Monocytes	10	16	18	16	13	11	11	7	12.8	1.2
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

TABLE A9.9A

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS)
OF MICE IN 'IMMUNIZED/CONJOINT' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	79	81	74	74	82	80	70	86	78.3	1.7
Neutrophils	10	11	10	18	8	13	18	11	12.4	1.2
Eosinophils	4	1	8	4	4	1	3	1	3.3	0.8
Monocytes	7	7	8	4	6	6	9	2	6.1	0.7
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	63	88	64	53	84	59	73	52	67.0	4.5
Neutrophils	28	8	25	20	15	40	22	42	25.0	3.8
Eosinophils	0	1	5	11	0	1	1	3	2.8	1.2
Monocytes	9	3	6	16	1	0	4	3	5.3	1.7
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	14	59	68	36	39	26	27	42	38.9	5.9
Neutrophils	67	31	17	53	38	56	61	20	42.9	6.3
Eosinophils	2	0	4	4	3	5	0	10	3.5	1.1
Monocytes	17	9	11	7	20	13	12	28	14.6	2.3
Basophils	0	1	0	0	0	0	0	0	0.1	0.1

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	32		44		71	38	50	72	51.2	6.3
Neutrophils	32		32		16	22	34	11	24.5	3.6
Eosinophils	1		1		2	7	0	6	2.8	1.1
Monocytes	35		23		11	33	16	11	21.5	4.0
Basophils	0		0		0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes			31		24	12	14		20.3	3.8
Neutrophils			42		43	53	37		43.8	2.9
Eosinophils			2		6	8	6		5.5	1.1
Monocytes			25		27	27	43		30.5	3.6
Basophils			0		0	0	0		0.0	0.0

TABLE A9.9B

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS)
OF MICE IN 'IMMUNIZED/NEMATODE' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	82	80	87	70	87	76	75	63	77.5	2.7
Neutrophils	13	15	11	20	5	11	12	25	14.0	2.0
Eosinophils	4	2	2	2	5	7	5	3	3.8	0.6
Monocytes	1	3	0	8	3	6	8	9	4.8	1.1
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	55	42	62	56	51	34	55	47	50.3	3.0
Neutrophils	37	46	36	27	49	41	42	41	39.9	2.2
Eosinophils	3	1	1	0	0	4	0	1	1.3	0.5
Monocytes	4	11	1	17	0	21	3	10	8.4	2.5
Basophils	1	0	0	0	0	0	0	1	0.3	0.2

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	45	48	63	31	76	61	55	65	55.5	4.6
Neutrophils	38	36	14	41	16	22	28	31	28.3	3.3
Eosinophils	8	11	11	11	1	6	5	2	6.9	1.3
Monocytes	9	5	12	17	7	11	12	2	9.4	1.6
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	49	53	59	51	73	69	74	60	61.0	3.3
Neutrophils	34	31	13	26	18	11	9	13	19.4	3.2
Eosinophils	9	9	25	14	3	12	11	14	12.1	2.1
Monocytes	8	7	3	9	6	8	6	13	7.5	1.0
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	57	55	59	49	47	37	63	42	51.1	2.9
Neutrophils	22	31	17	37	27	41	19	32	28.3	2.8
Eosinophils	10	7	19	8	12	13	10	15	11.8	1.3
Monocytes	11	7	5	6	14	9	8	11	8.9	1.0
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

TABLE A9.9C

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS)
OF MICE IN 'IMMUNIZED/TRYPANOSOME' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	87	85	76	83	72	81	89	79	81.5	1.9
Neutrophils	3	7	14	8	15	14	3	9	9.1	1.6
Eosinophils	6	5	7	6	12	4	4	7	6.4	0.8
Monocytes	4	3	3	3	1	1	4	5	3.0	0.5
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	63	70	74	76	79	62	87	87	74.8	3.2
Neutrophils	27	28	20	19	18	32	12	10	20.8	2.6
Eosinophils	0	0	2	0	0	0	0	0	0.3	0.2
Monocytes	10	2	4	5	3	6	1	3	4.3	0.9
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	75	43	74	63	53	49	59	72	61.0	4.0
Neutrophils	10	38	18	22	29	30	25	12	23.0	3.1
Eosinophils	3	1	1	8	4	3	3	2	3.1	0.7
Monocytes	12	18	7	7	14	18	13	14	12.9	1.4
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	45	52	38	62	40	62	45	52	49.5	3.0
Neutrophils	22	25	23	18	7	14	24	31	20.5	2.4
Eosinophils	6	0	3	3	3	1	3	3	2.8	0.6
Monocytes	27	23	36	17	50	23	28	14	27.3	3.8
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	27	34	29	29	25	50	37		33.0	3.0
Neutrophils	35	38	39	36	29	17	24		31.1	2.9
Eosinophils	5	2	5	3	2	1	1		2.7	0.6
Monocytes	33	26	27	32	44	32	38		33.1	2.2
Basophils	0	0	0	0	0	0	0		0.0	0.0

TABLE A9.9G

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS)
OF MICE IN 'UNIMMUNIZED/TRYPANOSOME' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	94	96	71	90	86	78	86	76	84.6	2.9
Neutrophils	4	3	23	8	6	14	8	19	10.6	2.4
Eosinophils	1	0	3	2	5	5	3	4	2.9	0.6
Monocytes	1	1	3	0	3	3	3	1	1.9	0.4
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	87	78	77	76	80	81	82	83	80.5	1.2
Neutrophils	9	16	22	17	10	12	14	11	13.9	1.4
Eosinophils	1	1	0	0	1	0	1	1	0.6	0.2
Monocytes	3	5	1	7	8	6	3	5	4.8	0.8
Basophils	0	0	0	0	1	1	0	0	0.3	0.2

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	48	43	48		58		56	55	51.3	1.9
Neutrophils	24	35	19		15		10	17	20.0	2.8
Eosinophils	0	1	3		1		2	4	1.8	0.5
Monocytes	28	21	30		26		32	24	26.8	1.3
Basophils	0	0	0		0		0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes		45						65	55.0	7.1
Neutrophils		41						16	28.5	8.8
Eosinophils		1						1	1.0	0.0
Monocytes		13						16	14.5	1.1
Basophils		0						0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes		26						36	31.0	3.5
Neutrophils		27						13	20.0	4.9
Eosinophils		0						1	0.5	0.4
Monocytes		47						50	48.5	1.1
Basophils		0						0	0.0	0.0

TABLE A9.9H

DIFFERENTIAL PERIPHERAL LEUCOCYTE COUNTS (PER 100 CELLS)
OF MICE IN 'UNIMMUNIZED/CONTROL' MICE IN CHAPTER NINE

Day 0	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	84	80	84	86	85	79	83	73	81.8	1.4
Neutrophils	7	17	14	10	14	16	9	19	13.3	1.4
Eosinophils	5	1	0	1	1	1	4	0	1.6	0.6
Monocytes	4	2	2	3	0	4	4	7	3.3	0.7
Basophils	0	0	0	0	0	0	0	1	0.1	0.1

Day 7	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	78	77	88	72	73	83	80	68	77.4	2.1
Neutrophils	12	14	8	22	15	14	14	27	15.8	2.0
Eosinophils	3	2	0	0	1	0	0	1	0.9	0.4
Monocytes	7	7	4	6	11	3	6	4	6.0	0.8
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 14	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	57	73	87	75	80	82	76	66	74.5	3.1
Neutrophils	23	17	8	16	13	10	21	26	16.8	2.1
Eosinophils	13	7	3	5	2	4	1	5	5.0	1.2
Monocytes	7	3	2	4	5	4	2	3	3.8	0.6
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 21	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	79	81	87	79	84	66	86	62	78.0	3.0
Neutrophils	4	13	5	17	13	30	12	30	15.5	3.3
Eosinophils	13	4	5	1	1	1	0	3	3.5	1.4
Monocytes	4	2	3	3	2	3	2	5	3.0	0.4
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

Day 28	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Lymphocytes	55	66	58	73	55	46	66	41	57.5	3.5
Neutrophils	25	21	26	19	29	39	27	55	30.1	3.9
Eosinophils	11	9	7	3	6	9	1	2	6.0	1.2
Monocytes	9	4	9	5	10	6	6	2	6.4	0.9
Basophils	0	0	0	0	0	0	0	0	0.0	0.0

TABLE A9.10A

ELISA VALUES (450 nm) AGAINST *H. POLYGYRUS*-DERIVED ANTIGENS FOR MICE USED IN CHAPTER NINE

IMMUNIZED/CONJOINT [Factor=1.0477767286]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.045	0.048	0.023	0.089	0.043	0.017	0.051	0.047	0.043	0.01
7	0.217	0.178	0.325	0.350	0.251	0.144	0.293	0.268	0.253	0.02
14	0.283	0.195	0.548	0.447	0.228	0.119	0.218	0.326	0.295	0.05
21	0.287		-0.062		-0.062	0.074	0.187	0.225	0.105	0.05
28			-0.062		-0.062	0.066	0.148		0.023	0.04

IMMUNIZED/NEMATODE [Factor=1.0281602971]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.037	-0.008	0.053	0.001	0.060	0.071	0.059	0.065	0.043	0.01
7	0.317	0.355	0.215	0.238	0.245	0.258	0.221	0.318	0.271	0.02
14	0.424	0.446	0.484	0.209	0.303	0.297	0.433	0.353	0.394	0.03
21	0.178	0.356	0.136	0.141	0.249	0.184	0.128	0.430	0.223	0.04
28	0.712	0.359	0.697	0.421	0.936	0.393	0.836	0.399	0.594	0.08

IMMUNIZED/TRYPANOSOME [Factor=1.1134383378]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.086	0.042	0.009	0.139	0.084	0.079	0.080	0.083	0.070	0.01
7	0.080	0.220	0.177	0.274	0.242	0.235	0.205	0.114	0.194	0.02
14	0.234	0.248	0.238	0.274	0.243	0.259	0.254	0.135	0.235	0.01
21	0.077	0.415	0.229	0.251	0.199	0.205	0.148	0.066	0.199	0.04
28	0.093	0.217	0.057	0.062	0.143	0.055	0.098		0.103	0.02

IMMUNIZED/CONTROL [Factor=1.0832]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.115	0.019	0.033	0.011	0.038	0.008	0.008	0.044	0.034	0.01
7	0.134	0.062	0.155	0.027	0.051	0.058	0.048	0.050	0.073	0.02
14	0.151	0.105	0.145	0.075	0.182	0.105	0.082	0.176	0.123	0.01
21	0.405	0.300	0.613	0.416	0.864	0.242	0.539	0.311	0.436	0.05
28	0.192	0.352	0.184	0.071	0.252	0.225	0.143	0.330	0.218	0.03

UNIMMUNIZED/CONJOINT [Factor=1.2068652379]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.028	0.004	0.282	0.058	0.003	-0.016	-0.007	0.003	0.044	0.03
7	0.032	0.136	0.206	0.150	0.004	0.038	0.128	0.075	0.096	0.02
14		0.096	0.086	0.130		0.093	0.084	0.047	0.090	0.01
21		0.029	0.015	0.075		0.059	0.049		0.045	0.01
28		0.088	0.064	0.151		0.079	0.105		0.098	0.01

UNIMMUNIZED/NEMATODE [Factor=1.1609014875]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.021	-0.021	-0.011	-0.022	-0.026	-0.020	-0.012	-0.026	-0.015	0.01
7	0.018	-0.015	0.019	0.033	0.020	0.005	0.013	-0.016	0.010	0.01
14	0.042	0.015	0.060	0.145	0.055	0.086	0.105	0.037	0.086	0.01
21	0.029	-0.005	0.064	0.060	0.065	0.076	0.091	0.061	0.055	0.01
28	0.163	0.010	0.154	0.133	0.198	0.139	0.182	0.184	0.145	0.02

UNIMMUNIZED/TRYPANOSOME [Factor=1.3608437436]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.038	0.015	-0.008	-0.015	-0.007	-0.022	0.001	-0.001	0.000	0.01
7	0.016	-0.019	-0.022	-0.007	-0.002	-0.015	0.005	-0.007	-0.007	0.00
14	-0.003	-0.004	-0.038		-0.021		0.013	0.032	-0.003	0.01
21		0.032						0.025	0.029	0.00
28		0.037						0.039	0.038	0.00

UNIMMUNIZED/CONTROL [Factor=1.3003813894]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.045	0.001	-0.008	0.004	-0.001	-0.017	-0.009	0.005	0.003	0.01
7	0.019	-0.035	-0.012	-0.005	-0.021	-0.008	-0.008	0.024	-0.006	0.01
14	0.007	-0.023	0.003	0.034	-0.011	0.008	0.010	0.039	0.008	0.01
21	0.013	-0.016	-0.007	0.014	-0.010	0.021	0.008	0.033	0.007	0.01
28	0.019	-0.020	-0.007	0.020	-0.008	0.018	0.014	0.046	0.010	0.01

TABLE A9.10B

ELISA VALUES (450 nm) AGAINST *T. CONGOLENSE*-DERIVED ANTIGENS FOR MICE USED IN CHAPTER NINE

IMMUNIZED/CONJOINT [Factor=1.0399658655]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.014	0.063	-0.031	-0.040	-0.048	-0.035	0.002	-0.004	-0.010	0.01
7	0.128	0.260	0.040	0.212	0.364	0.219	0.284	0.587	0.262	0.05
14	0.401	0.685	0.422	0.363	1.081	0.735	0.847	1.060	0.699	0.10
21	0.811		0.331		1.327	1.034	1.227	1.403	0.989	0.16
28			0.912		1.372	1.241	1.321		1.211	0.09

IMMUNIZED/NEMATODE [Factor=1.0827780246]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.015	-0.003	-0.005	-0.042	-0.026	-0.019	0.008	0.011	-0.008	0.01
7	0.193	-0.022	0.140	0.131	0.118	0.052	0.019	0.082	0.087	0.02
14	-0.002	0.075	0.059	0.000	0.218	-0.003	-0.016	0.063	0.049	0.03
21	-0.060	-0.036	-0.060	-0.033	-0.052	-0.059	-0.053	-0.029	-0.048	0.00
28	0.000	-0.044	-0.022	-0.018	0.139	-0.008	-0.009	-0.002	0.005	0.02

IMMUNIZED/TRYPANOSOME [Factor=1.0787843022]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.004	0.007	0.038	-0.046	-0.018	-0.020	-0.019	-0.020	-0.010	0.01
7	0.018	-0.050	0.128	-0.035	0.023	0.013	-0.006	0.047	0.017	0.02
14	-0.004	-0.027	0.105	0.118	0.091	0.078	0.091	0.083	0.087	0.02
21	-0.038	-0.034	0.082	-0.008	0.019	0.013	0.044	0.038	0.015	0.01
28	-0.025	-0.053	0.078	0.010	0.001	0.012	0.048	0.098	0.021	0.02

IMMUNIZED/CONTROL [Factor=1.1005418423]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.037	-0.028	-0.043	-0.079	-0.037	-0.065	-0.063	-0.028	-0.038	0.01
7	-0.039	-0.049	-0.017	-0.053	-0.056	-0.047	-0.042	-0.048	-0.044	0.00
14	-0.064	-0.064	-0.049	-0.081	-0.055	-0.049	-0.088	-0.054	-0.058	0.00
21	-0.038	-0.032	0.004	-0.001	0.119	-0.001	0.021	0.062	0.017	0.02
28	-0.054	-0.049	-0.029	-0.047	-0.066	-0.048	-0.057	-0.055	-0.051	0.00

UNIMMUNIZED/CONJOINT [Factor=1.1070401211]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.002	-0.055	-0.040	-0.005	-0.026	-0.001	-0.030	-0.006	-0.021	0.01
7	0.342	0.300	0.188	0.350	0.376	0.389	0.288	0.216	0.306	0.02
14		0.638	0.808	0.845		1.097	0.462	0.201	0.675	0.12
21		0.435	0.871	0.904		1.050	0.831		0.818	0.09
28		0.852	1.174	1.234		1.153	1.027		1.088	0.06

UNIMMUNIZED/NEMATODE [Factor=1.0787843022]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.004	0.007	0.038	-0.046	-0.018	-0.020	-0.019	-0.020	-0.010	0.01
7	0.018	-0.050	0.128	-0.035	0.023	0.013	-0.006	0.047	0.017	0.02
14	-0.004	-0.027	0.105	0.118	0.091	0.078	0.091	0.083	0.087	0.02
21	-0.038	-0.034	0.082	-0.008	0.019	0.013	0.044	0.038	0.015	0.01
28	-0.025	-0.053	0.078	0.010	0.001	0.012	0.048	0.098	0.021	0.02

UNIMMUNIZED/TRYPANOSOME [Factor=1.0799793221]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	-0.013	-0.035	-0.043	-0.057	-0.020	-0.043	-0.013	-0.037	-0.033	0.01
7	0.282	0.183	0.235	0.208	0.204	0.182	0.292	0.299	0.236	0.02
14	0.437	0.726	0.080		0.130		1.049	1.080	0.584	0.16
21		0.809						0.816	0.813	0.00
28		1.102						1.010	1.056	0.03

UNIMMUNIZED/CONTROL [Factor=1.1408956155]

Days	M1	M2	M3	M4	M5	M6	M7	M8	MEAN	SE
0	0.075	0.083	-0.014	-0.020	0.000	0.002	-0.039	-0.007	0.010	0.01
7	0.011	-0.039	-0.038	-0.027	-0.031	-0.045	-0.031	-0.007	-0.026	0.01
14	0.010	-0.040	-0.038	-0.038	-0.030	-0.033	-0.034	-0.009	-0.026	0.01
21	-0.017	-0.044	-0.034	-0.038	-0.022	0.009	-0.030	0.015	-0.020	0.01
28	-0.014	-0.047	-0.042	-0.037	-0.022	-0.006	-0.024	0.022	-0.021	0.01

TABLE A10.1A

T. CONGOLENSE PARASITAEMIAS OF SURVIVING MICE IN GROUP 'ITH/0' OF CHAPTER TEN

ITH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.1	8
6	6.0	6.3	5.7	6.3	6.0	6.6	6.2	6.5	6.2	0.2	8
7	6.6	7.2	6.0	7.2	6.6	7.8	6.9	7.5	7.0	0.2	8
8	7.1	7.7	6.6	7.7	6.6	7.9	6.8	7.8	7.2	0.2	8
9	7.5	8.1	7.2	8.1	6.6	8.0	6.6	8.0	7.5	0.2	8
10	7.5	8.4	7.2	8.4	6.6	8.0	6.6	8.1	7.6	0.1	8
11	7.8	7.5	7.6	7.7	7.3	7.9	7.1	6.6	7.4	0.3	8
12	8.0	6.6	7.9	6.9	7.9	7.8	7.5	5.0	7.2	0.2	8
13	8.0	7.4	8.0	7.6	7.6	7.8	7.7	6.1	7.5	0.1	8
14	8.0	8.2	8.1	8.2	7.2	7.8	7.8	7.2	7.8	0.1	8
15		8.2	8.1	8.1	7.2	8.0	7.9	7.4	7.8	0.1	7
16		8.2	8.1	8.0	7.2	8.1	8.0	7.5	7.9	0.1	7
17		8.2	8.1	7.9	7.2	8.1	8.0	7.5	7.9	0.1	7
18		8.2	8.1	8.0	7.2	8.1	8.0	7.5	7.9	0.1	7
19		8.1	8.1	8.1	7.2	8.1	8.0	7.5	7.9	0.1	7
20		8.2	8.1	8.3	7.4		7.0	7.5	7.7	0.2	6
21		8.2	8.1	8.4	7.5		6.0	7.5	7.6	0.3	6
22		8.2	8.1	8.2	7.5		6.8	7.5	7.7	0.2	6
23		8.2	8.1	8.0	7.5		7.5	7.5	7.8	0.1	6
24		8.2	8.0	7.8	7.2		7.5	7.5	7.7	0.1	6
25		8.2	7.9	8.1	7.5		7.5	7.7	7.8	0.1	6
26		8.2	7.8	8.3	7.8		7.5	7.9	7.9	0.1	6
27		8.0	7.5	8.3	7.9		7.4	7.9	7.8	0.1	6
28		7.8	7.2	8.3	8.0		7.2	7.8	7.7	0.2	6
29		8.3	7.8	8.1	7.2		5.7	6.3	7.2	0.4	6

TABLE A10.1B

T. CONGOLENSE PARASITAEMIAS OF SURVIVING MICE IN GROUP 'ITH/10' OF CHAPTER TEN

ITH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
6	6.5	6.0	6.3	6.3	6.3	6.3	6.2	6.5	6.3	0.1	8
7	7.5	6.6	7.2	7.2	7.2	7.2	6.9	7.5	7.2	0.1	8
8	7.9	7.2	7.7	7.8	7.7	7.6	7.5	7.8	7.6	0.1	8
9	8.2	7.8	8.1	8.3	8.1	7.9	8.1	8.1	8.1	0.1	8
10	8.7	7.8	8.4	8.7	8.4	8.0	8.4	8.1	8.3	0.1	8
11	8.4	7.8	8.0	8.6	8.5	7.6	7.8	8.1	8.1	0.2	8
12	8.1	7.8	7.5	8.4	8.5	7.2	7.2	8.1	7.9	0.1	8
13	8.1	7.7	7.9	8.4	8.6	7.7	7.7	8.1	8.0	0.1	8
14		7.5	8.2	8.4	8.6	8.1	8.1	8.1	8.1	0.1	7
15		7.8	8.2	8.3	8.5	8.1	8.0	8.1	8.1	0.1	7
16		8.1	8.1	8.1	8.3	8.1	7.9	8.1	8.1	0.0	7
17		8.2	8.1	7.9	8.3	8.2	7.9	8.2	8.1	0.1	7
18		8.2	8.2	8.1		8.2	8.4	8.1	8.2	0.1	6
19		8.1	8.2	8.3		8.2	8.9		8.3	0.1	5
20		8.1	8.2	8.2		8.2	8.8		8.3	0.1	5
21		8.1		8.1		8.1	8.6		8.2	0.1	4
22		7.9		8.1		8.1	8.6		8.2	0.1	4
23		7.7		8.1		8.1	8.6		8.1	0.2	4
24		7.5				8.1			7.8	0.2	2
25		7.8							7.8	0.0	1
26		8.1							8.1	0.0	1
27											1
28											0

BLE A10.1C

CONGOLENSE PARASITAEMIAS OF SURVIVING MICE IN GROUP 'IT' CHAPTER TEN

ys	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
6	6.5	6.3	6.6	6.6	6.3	6.5	6.5	6.5	6.5	0.0	8
7	7.5	7.2	7.8	7.8	7.2	7.5	7.5	7.5	7.5	0.1	8
8	8.0	7.7	8.0	8.0	7.4	7.8	7.8	7.5	7.7	0.1	8
9	8.4	8.1	8.1	8.1	7.5	8.1	8.1	7.5	8.0	0.1	8
10	8.9	8.4	8.1	8.4	7.8	8.3	8.3	7.5	8.2	0.1	8
11	8.8	8.2	8.1	8.4	7.9	8.1	8.1	7.5	8.1	0.1	8
12	8.7	7.9	8.0	8.4	7.9	7.9	7.9	7.5	8.0	0.1	8
13	8.4	7.6	8.1	8.5	8.3	8.1	8.1	7.7	8.1	0.1	8
14	8.1	7.2	8.1	8.5	8.7	8.3	8.2	7.8	8.1	0.2	8
15	8.1	7.2	8.1	8.3	8.5	8.3		7.8	8.0	0.1	7
16	8.1	7.2	8.1	8.1	8.3	8.2		7.8	8.0	0.1	7
17			8.1	7.9	7.8	8.2		7.8	8.0	0.1	5
18			8.0	7.7	7.8	8.1		8.0	7.9	0.1	5
19			7.8	7.5	7.8	8.0		8.1	7.8	0.1	5
20			7.9	7.9	7.9	7.8		7.8	7.8	0.0	5
21			7.9	8.2	7.9	7.5		7.5	7.8	0.1	5
22			8.0	8.2	7.9	7.5		7.7	7.9	0.1	5
23			8.1	8.2	7.9	7.5		7.8	7.9	0.1	5
24			8.3	8.2	7.8	7.2		7.8	7.9	0.2	5
25			8.3	8.2	7.7	7.7		8.0	8.0	0.1	5
26			8.3	8.1	7.5	8.2		8.1	8.0	0.1	5
27			8.2	8.0	7.4	7.9		7.7	7.8	0.1	5
28			8.1	7.9	7.2	7.5		7.2	7.6	0.1	5
29			7.9	6.9	6.9	7.5		7.2	7.3	0.2	5

TABLE A10.1D

T. CONGOLENSE PARASITAEMIAS OF SURVIVING MICE IN GROUP 'UTH/10' OF CHAPTER TEN

UTH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE	Survivors
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
5	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.0	8
6	6.5	6.3	6.5	6.3	6.3	6.5	6.5	6.5	6.4	0.1	8
7	7.5	7.2	7.5	7.2	7.2	7.5	7.5	7.5	7.4	0.1	8
8	8.1	7.7	7.8	7.8	7.7	7.8	7.8	8.1	7.9	0.1	8
9	8.7	8.2	8.1	8.4	8.2	8.1	8.1	8.7	8.3	0.1	8
10	8.7	8.0	8.1	8.4	8.3	8.1	8.3	8.8	8.3	0.1	8
11		7.8	8.1	8.4	8.4	8.1	8.4	8.9	8.3	0.1	7
12		8.0	8.0	8.2		8.3	8.3	8.8	8.2	0.1	6
13		8.2	7.8	7.9		8.4	8.1		8.1	0.0	5
14		8.2	8.0	8.0			8.1		8.1	0.0	4
15		8.1	8.1	8.1			8.1		8.1	0.0	4
16		7.7	8.1	8.1			7.9		8.0	0.1	4
17											0

TABLE A10.2

THE PCVs (%) OF MICE USED IN CHAPTER TEN

ITH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	52	53	50	50	51	50	48	50.6	0.5
7	50	48	53	46	48	50	52	48	49.4	0.8
14	30	42	43	38	38	31	40	42	38.0	1.6
21		30	40	38	34		41	37	36.7	1.5
28		32	50	40	36		44	40	40.3	2.3

UTH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	51	51	51	54	48	51	53	51.1	0.6
7	46	40	47	46	46	43	48	48	45.5	0.9
14		28	26	28		30	32		28.8	0.9
21										
28										

ITH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	54	53	50	50	52	52	49	53	51.6	0.6
7	45	53	46	48	50	48	48	48	48.3	0.8
14		34	45	40	29	44	29	34	36.4	2.3
21		37		34		31	36		34.5	1.0
28		22							22.0	0.0

UH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	54	50	49	52	52	53	54	51.8	0.6
7	46	49	51	42	47	49	45	54	47.9	1.2
14	50	53	48	49	46	47	49	48	48.8	0.7
21	48	54	50	51	50	50	52	52	50.9	0.6
28	50	54	54	52	50	53	54	47	51.8	0.8

IH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	53	51	51	51	49	50	51	50.9	0.4
7	51	52	49	50	51	50	51	51	50.6	0.3
14	50	53	50	51	48	47	49	48	49.5	0.6
21	50	48	38	41	40	34	45	47	42.9	1.8
28	44	50	45	47	49	48	46	51	47.5	0.8

IC

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	52	54	48	50	53	50	49	50.8	0.7
7	48	45	47	46	48	53	52	49	48.5	0.9
14	49	48	52	46	50	50	50	50	49.4	0.6
21	46	46	48	45	46	50	51	52	48.0	0.9
28	49	45	51	47	50	51	48	47	48.5	0.7

IH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	51	54	52	52	52	53	54	51	52.4	0.4
7	47	48	47	45	47	50	45	44	46.6	0.6
14	47	49	45	46	45	46	45	44	45.9	0.5
21	50	48	46	48	46	48	50	48	48.0	0.5
28	50	49	52	46	50	50	52	48	49.6	0.7

UC

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	49	48	52	50	52	50	51	47	49.9	0.6
7	44	48	52	51	52	49	50	48	49.3	0.9
14	46	49	51	48	50	49	51	52	49.5	0.6
21	50	48	52	54	52	52	48	47	50.4	0.8
28	47	49	47	50	54	49	50	46	49.0	0.8

IT

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	50	54	52	51	55	50	50	50	51.5	0.7
7	47	47	46	44	48	45	47	46	46.3	0.4
14	27	15	36	36	40	26	38	39	32.1	2.9
21			41	40	38	36		36	38.2	0.9
28			38	36	39	39		40	38.4	0.6

TABLE A10.3

THE WEIGHTS (g) OF MICE USED IN CHAPTER TEN

ITH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	25	27	26	29	28	26	25	28	26.8	0.5
7	25	28	28	30	27	26	25	28	27.1	0.6
14	26	28	27	29	28	25	24	28	26.9	0.6
21		34	30	34	30		28	31	31.2	0.9
28		35	30	35	34		29	35	33.0	1.0

UTH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	27	25	27	24	27	27	25	26.1	0.4
7	28	33	27	28	25	28	29	28	28.3	0.7
14		32	26	27		26	29		28.0	1.0
21										
28										

ITH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	24	26	27	23	28	25	32	28	26.6	0.9
7	26	26	28	25	29	25	32	29	27.5	0.8
14		26	25	24	28	24	33	25	26.4	1.1
21		28		26		28	35		29.3	1.5
28		23							23.0	0.0

UH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	25	26	26	26	28	27	27	26.5	0.3
7	29	27	27	28	28	31	27	28	28.1	0.4
14	28	26	26	27	28	32	27	28	27.8	0.6
21	31	27	29	28	31	31	30	27	29.3	0.6
28	32	27	27	28	30	33	30	29	29.5	0.7

IH/10

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	27	25	27	27	26	29	25	26.6	0.4
7	28	30	26	30	30	30	30	27	28.9	0.5
14	27	28	25	27	27	28	28	24	26.8	0.5
21	26	32	29	29	29	26	29	28	28.5	0.6
28	29	32	28	32	31	31	33	28	30.5	0.6

IC

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	26	28	29	25	27	25	27	26.8	0.5
7	30	31	31	32	30	29	26	28	29.6	0.6
14	28	32	32	30	28	28	27	26	28.9	0.7
21	31	29	33	30	30	31	28	29	30.1	0.5
28	31	31	32	33	29	31	29	27	30.4	0.6

IH/0

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	29	23	26	26	28	28	24	26.4	0.7
7	28	28	25	28	27	30	29	26	27.6	0.5
14	28	27	25	26	25	29	30	27	27.1	0.6
21	30	28	27	27	29	31	32	27	28.9	0.6
28	33	30	29	30	29	32	33	28	30.5	0.6

UC

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	25	25	28	27	28	27	24	24	26.0	0.6
7	29	27	31	28	30	28	27	26	28.3	0.6
14	26	25	29	28	30	28	25	25	27.0	0.7
21	27	26	30	29	33	28	27	27	28.4	0.7
28	28	25	30	29	33	29	27	28	28.6	0.8

IT

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	27	26	26	26	26	27	29	28	26.9	0.4
7	27	28	28	28	28	29	30	30	28.5	0.4
14	25	25	32	31	30	28	30	32	29.1	0.9
21			31	32	33	30		37	32.6	1.1
28			34	36	35	35		40	36.0	0.9

TABLE A10.4

ESTIMATED INITIAL AND FINAL EVISCERATED CARCASS WEIGHTS (g) OF MICE USED IN CHAPTER TEN

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
ITH/0										
Terminal		16	19	19	18		17	18	17.8	0.4
Starting		20.4	19.7	22.0	21.2		18.9	21.2	20.6	0.4
Difference		-4.4	-0.7	-3.0	-3.2		-1.9	-3.2	-2.7	0.5
ITH/10										
Terminal		10							10.0	0.0
Starting		19.7							19.7	0.0
Difference		-9.7							-9.7	0.0
IH/10										
Terminal	20	21	19	21	19	20	22	18	20.0	0.4
Starting	20.4	20.4	18.9	20.4	20.4	19.7	22.0	18.9	20.2	0.3
Difference	-0.4	0.6	0.1	0.6	-1.4	0.3	0.0	-0.9	-0.2	0.2
IH/0										
Terminal	21	21	19	21	20	22	22	19	20.6	0.4
Starting	20.4	22.0	17.4	19.7	19.7	21.2	21.2	18.2	20.0	0.5
Difference	0.6	-1.0	1.6	1.3	0.3	0.8	0.8	0.8	0.7	0.3
IT										
Terminal			17	20	22	20		24	20.6	1.0
Starting			19.7	19.7	19.7	20.4		21.2	20.1	0.3
Difference			-2.7	0.3	2.3	-0.4		2.8	0.5	0.9
UH/0										
Terminal	21	20	20	21	21	23	21	20	20.9	0.3
Starting	20.4	18.9	19.7	19.7	19.7	21.2	20.4	20.4	20.1	0.2
Difference	0.6	1.1	0.3	1.3	1.3	1.8	0.6	-0.4	0.8	0.2
IC										
Terminal	23	23	25	24	22	23	22	20	22.8	0.5
Starting	20.4	19.7	21.2	22.0	18.9	20.4	18.9	20.4	20.2	0.3
Difference	2.6	3.3	3.8	2.0	3.1	2.6	3.1	-0.4	2.5	0.4
UC										
Terminal	22	19	22	22	25	23	20	22	21.9	0.6
Starting	18.9	18.9	21.2	20.4	21.2	20.4	18.2	18.2	19.7	0.4
Difference	3.1	0.1	0.8	1.6	3.8	2.6	1.8	3.8	2.2	0.5

TABLE A10.5

THE WORM BURDENS OF MICE USED IN CHAPTER NINE

ITH/0

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males		26	4	70	0		54	0	25.7	11
Females		10	14	47	1		44	1	19.5	8
Total		36	18	117	1		98	1	45.2	19
%Protection		87.5	93.7	59.2	99.7		65.8	99.7	84.3	7

ITH/10

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males		54							54.0	0
Females		80							80.0	0
Total		134							134.0	0
%Protection		53.3							53.3	0

IH/0

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males	0	0	112	0	0	0	0	0	14.0	13
Females	0	0	89	0	0	0	0	0	11.1	10
Total	0	0	201	0	0	0	0	0	25.1	24
%Protection	100.0	100.0	29.9	100.0	100.0	100.0	100.0	100.0	91.2	8

IH/10

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males	0	0	0	0	7	2	28	0	4.6	3
Females	0	0	0	0	63	8	55	0	15.8	9
Total	0	0	0	0	70	10	83	0	20.4	12
%Protection	100.0	100.0	100.0	100.0	75.6	96.5	71.1	100.0	92.9	4

UH/0

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
Males	191	145	134	126	143	108	163	93	137.9	10
Females	167	120	149	112	166	140	202	136	149.0	10
Total	358	265	283	238	309	248	365	229	286.9	17
%Protection	-24.8	7.6	1.4	17.0	-7.7	13.6	-27.2	20.2	0.0	6

TABLE A10.8A

THE ELISA VALUES (450 nm) AGAINST *H. POLYGYRUS*-DERIVED ANTIGENS
FOR MICE USED IN CHAPTER TEN

ITH/0 [Factor=9.3067226891]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.290	0.155	0.202	0.155	0.189	0.088	0.072	0.181	0.160	0.02
7	0.774	0.342	0.421	0.477	0.356	0.337	0.374	0.495	0.447	0.05
14	0.818	0.397	0.397	0.383	0.458	0.421	0.207	0.570	0.431	0.04
21		0.267	0.369	0.342	0.653		0.314	0.523	0.411	0.05
28		0.300	0.421	0.332	0.528		0.304	0.504	0.398	0.04

ITH/10 [Factor=8.65234375]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.202	0.193	0.178	0.154	0.318	0.266	0.072	0.163	0.193	0.02
7	0.215	0.431	0.258	0.167	0.509	0.262	0.301	0.418	0.320	0.04
14		0.379	0.167	0.271	0.323	0.409	0.392	0.405	0.335	0.03
21		0.565		0.236		0.362	0.279		0.361	0.06
28		0.578							0.578	0.00

IM/10 [Factor=9.0285326087]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.303	0.249	0.222	0.321	-0.031	-0.053	0.023	0.109	0.143	0.05
7	0.019	-0.026	-0.076	-0.062	-0.098	-0.049	-0.085	0.114	-0.033	0.02
14	0.574	0.321	0.610	0.800	0.272	0.317	0.281	0.655	0.479	0.07
21	0.822	0.497	0.868	0.637	0.371	0.294	0.335	0.651	0.560	0.07
28	0.908	0.958	1.513	1.328	0.497	0.534	0.777	1.391	0.988	0.13

IM/0 [Factor=10.5812101911]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.424	0.139	0.033	0.229	0.123	0.266	0.022	0.149	0.173	0.04
7	0.419	0.382	0.572	0.726	0.080	0.128	0.086	0.557	0.389	0.08
14	1.017	0.504	0.424	1.075	0.996	1.165	0.726	1.086	0.874	0.09
21	0.816	0.832	0.313	0.890	0.906	1.276	0.742	1.191	0.871	0.10
28	0.828	0.842	0.276	1.070	0.879	1.117	0.953	1.594	0.945	0.12

IT [Factor=1.2609108159]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.237	0.129	0.187	0.254	0.200	0.155	0.240	0.145	0.193	0.02
7	0.354	0.182	0.230	0.317	0.211	0.148	0.260	0.250	0.244	0.02
14	0.123	0.249	0.182	0.205	0.153	0.034	0.175	0.174	0.162	0.02
21			0.220	0.317	0.194	0.085		0.118	0.187	0.04
28			0.287	0.341	0.238	0.107		0.196	0.234	0.04

UTH/10 [Factor=1.4537300372]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.034	-0.002	-0.007	-0.015	-0.001	-0.018	-0.025	-0.030	-0.008	0.01
7	0.004	0.010	0.023	0.029	0.028	-0.022	-0.015	0.000	0.007	0.01
14		-0.025	-0.009	0.000		-0.007	-0.011		-0.010	0.00
21										
28										

UH/0 [Factor=1.4855932951]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.037	-0.002	-0.020	-0.038	-0.041	-0.021	-0.032	-0.016	-0.017	0.01
7	0.416	0.385	0.284	0.307	0.310	0.405	0.351	0.398	0.357	0.02
14	0.128	0.074	-0.002	0.008	0.046	0.055	-0.002	0.119	0.053	0.02
21	0.077	0.084	0.026	0.018	0.059	0.044	0.078	0.114	0.060	0.01
28	0.146	0.109	0.042	0.035	0.079	0.154	0.098	0.230	0.112	0.02

IC [Factor=1.8850014033]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.326	0.642	0.260	0.143	0.190	0.254	0.185	0.313	0.289	0.05
7	0.294	0.698	0.425	0.297	0.286	0.335	0.389	0.370	0.387	0.04
14	0.386	0.828	0.556	0.282	0.253	0.325	0.731	0.602	0.495	0.07
21	0.315	0.804	0.505	0.302	0.361	0.374	0.597	0.650	0.489	0.06
28	0.274	1.016	0.538	0.291	0.442	0.432	0.496	0.486	0.497	0.08

UC [Factor=1.5879565833]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.072	0.018	-0.001	0.030	-0.031	-0.043	-0.021	-0.008	0.002	0.01
7	0.045	0.001	-0.018	-0.009	-0.019	-0.018	-0.017	0.007	-0.003	0.01
14	0.027	-0.018	-0.004	0.016	-0.013	-0.003	-0.007	0.048	0.006	0.01
21	0.029	-0.014	-0.002	0.027	0.007	0.005	0.009	0.043	0.013	0.01
28	0.035	-0.016	0.005	-0.001	-0.009	0.009	0.009	0.040	0.009	0.01

TABLE A10.8B

THE ELISA VALUES (450 nm) AGAINST *T. CONGOLENSE*-DERIVED ANTIGENS
FOR MICE USED IN CHAPTER TEN

ITH/0 [Factor=7.0784123911]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	-0.042	-0.052	-0.052	0.033	-0.095	0.075	-0.021	0.156	0.000	0.03
7	0.785	0.344	0.574	0.648	0.309	0.588	0.588	0.503	0.540	0.05
14	0.825	0.924	0.694	0.758	0.992	0.978	0.740	0.878	0.849	0.04
21		0.985	0.981	0.733	0.978		0.914	0.875	0.911	0.04
28		1.250	1.501	1.183	1.314		1.328	1.576	1.359	0.06

ITH/10 [Factor=6.419666374]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	-0.042	-0.141	-0.087	-0.109	-0.188	-0.051	-0.183	-0.071	-0.109	0.02
7	0.363	0.128	0.266	0.205	0.263	0.263	-0.042	0.401	0.231	0.05
14		0.411	0.822	0.523	0.112	0.578	0.263	0.835	0.506	0.09
21		0.690		0.260		0.453	0.443		0.461	0.08
28		0.597							0.597	0.00

IM/10 [Factor=6.6654512308]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.115	0.025	0.001	0.035	-0.099	-0.085	-0.009	-0.015	-0.004	0.02
7	0.021	-0.042	-0.039	-0.005	-0.089	0.015	-0.059	0.148	-0.008	0.02
14	0.158	0.001	0.211	0.078	-0.059	-0.015	-0.035	0.218	0.070	0.04
21	0.298	0.188	0.101	0.265	0.021	0.251	0.171	0.391	0.211	0.04
28	0.168	0.101	0.171	0.331	0.031	0.255	0.348	0.371	0.222	0.04

IM/0 [Factor=8.265638389]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.123	-0.021	-0.008	-0.033	-0.077	-0.024	-0.080	-0.014	-0.017	0.02
7	0.136	0.033	0.014	0.070	-0.052	-0.052	-0.090	-0.033	0.003	0.02
14	0.340	-0.052	0.001	0.081	-0.065	0.133	-0.083	0.089	0.053	0.05
21	0.058	-0.058	-0.021	-0.033	-0.121	0.001	0.189	-0.002	0.002	0.03
28	0.083	-0.049	-0.024	0.001	-0.096	0.023	-0.043	0.014	-0.011	0.02

IT [Factor=1.0289895883]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.014	-0.028	0.036	0.052	0.087	0.040	-0.004	-0.036	0.020	0.01
7	0.497	0.274	0.464	0.456	0.445	0.292	0.313	0.384	0.390	0.03
14	0.574	0.500	0.621	0.876	1.050	0.357	0.843	0.467	0.661	0.08
21			0.910	1.099	1.369	1.056		0.583	1.004	0.12
28			1.130	1.255	1.439	1.285		1.011	1.224	0.06

UTH/10 [Factor=1.0131633643]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	-0.042	-0.041	-0.035	-0.002	0.010	-0.046	-0.055	-0.003	-0.027	0.01
7	0.236	0.303	0.291	0.366	0.454	0.164	0.251	0.288	0.294	0.03
14		0.339	0.285	0.297		0.316	0.336		0.315	0.01
21										
28										

UH/0 [Factor=1.065733858]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	0.065	0.007	-0.046	-0.062	-0.087	-0.059	-0.083	-0.023	-0.031	0.02
7	0.039	0.038	0.075	0.081	0.029	0.240	0.025	0.112	0.080	0.02
14	0.352	0.195	-0.044	-0.027	0.039	0.036	-0.032	0.231	0.094	0.05
21	0.142	0.069	-0.026	-0.024	0.008	-0.008	-0.017	0.120	0.033	0.02
28	0.099	0.099	-0.042	-0.030	-0.008	0.032	-0.021	0.114	0.030	0.02

IC [Factor=1.0156986245]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	-0.010	0.029	0.021	-0.060	0.023	0.086	-0.050	0.113	0.019	0.02
7	-0.032	0.002	-0.009	-0.069	-0.026	-0.014	-0.028	0.047	-0.016	0.01
14	-0.005	-0.007	0.027	-0.050	-0.039	-0.023	-0.003	0.051	-0.008	0.01
21	-0.007	-0.014	0.019	-0.059	-0.023	0.011	0.006	0.069	0.000	0.01
28	0.015	0.037	0.047	0.072	-0.016	0.056	0.038	0.003	0.032	0.01

UC [Factor=1.180878553]

Days	M1	M2	M3	M4	M5	M6	M7	M8	Mean	SE
0	-0.046	-0.065	-0.090	-0.054	-0.085	-0.095	-0.067	-0.090	-0.074	0.01
7	-0.042	-0.064	-0.128	0.004	-0.065	-0.102	-0.087	-0.064	-0.071	0.01
14	-0.039	-0.080	-0.083	0.026	-0.105	-0.088	-0.092	-0.008	-0.059	0.02
21	-0.035	-0.063	-0.097	0.017	-0.041	-0.089	-0.080	-0.045	-0.054	0.01
28	-0.006	-0.064	0.009	-0.057	-0.054	-0.079	-0.071	-0.041	-0.045	0.01

APPENDIX TWO

PREPARATION OF SOLUTIONS AND REAGENTS

PURIFIED WATER

The water used for preparing media and other solutions for tissue culture was purified by the Milli Ro 60 system. It was then given a final polishing with the Milli Q system (Millipore), such that the resultant resistivity was 18 mega ohn cm^{-2}, indicating an extremely high purity.

STERILISATION OF MATERIALS USED FOR TISSUE CULTURE

Purified waster was sterilised by heating to 121°C, under a pressure of 1.1kg cm^{-2} for 15-20 minutes. Phosphate buffered saline was also sterilised by this method. All solutions were sterilised by passing through a 0.22μm, low protein absorption, disposable filter (Millex GV, Millipore). Pipettes were sterilised in a dry heat oven at 170°C for 90 minutes.

PHOSPHATE BUFFERED SALINE (PBS)

Sodium chloride	10.11g
Potassium chloride	0.36 kg
Potassium dihydrogen phosphate	0.36g
Disodium hydrogen orthophosphate	0.36g
Make up with distilled water to 1 litre	1.45g

PHOSPHATE SALINE GLUCOSE (PSG)

Glucose	10.00g
Sodium chloride	2.58g
Disodium hydrogen orthophosphate (anhydrous)	8.10g
Sodium dihydrogen orthophosphate	8.10g

Dissolve in 1 litre of distilled water and adjust to pH 8.0

PREPARATION OF DE52 (WHATMAN) FOR SEPARATION OF TRYPANOSOMES

Suspend 500 g DE52 PSG. Allow to stand and remove "fines". Adjust to pH 8.0 with 5% ortophosphoric acid. Wash four times with 2000 ml PSG. Store at 4°C or at -20°C for long period-storage.

PREPARATION OF MINIMAL ESSENTIAL MEDIUM (EAGLE) - MEM - FROM POWDER (GIBCO CAT NO. 410-1100)

Measure 500 ml of double distilled water into sterile 2 litre beaker. Open packet of medium carefully and spread powder on top of water. Add rest of water, 30ml of 7.5% Sodium hydrogen carbonate (w/v - i.e. final concentration of 2.2g/l) and 20ml sterile 1M Hepes pH 7.3 (i.e. final concentration of 20mM). Filter through Millipore apparatus with 0.22μ filter and dispense into 100ml bottles.

HANKS BALANCE SALT SOLUTION (HBSS) WITH ANTIBIOTICS

HBSS	4.88g
Soidum bicarbonate	0.18g
Streptomycin solution (x100)	5.0ml
Fungizone (x500)	1.0ml
Distilled water	500ml

ELISA WASHING SOLUTION

Sodium chloride	9.0g
Tween 20	0.5g

Distilled water to 1 litre

Adjust to pH 8.2 with hydrochloric acid and store at 4°C

SODIUM ACETATE/CITRATE BUFFER

Solution A: 49.2g of sodium acetate (anhydrous) made up to 1 litre with distilled water (0.6M). Solution B: 52.5g of citric acid made to 500ml with distilled water (0.5M). x5 stock solution was prepared by adding 100ml of Solution B to 50ml of Solution A and adjusting to pH 6.0. Stock solution with diluted 1:5 for use, i.e.

200ml x5 Stock + 800ml of distilled water to give 0.1M acetate/citrate acid buffer pH 6.0.

TMB SUBSTATE

3,3',5,5'-Tetramethylbenzidine (TMB)	5mg
Dissolve in DMSO (Analar)	0.5ml
0.1M sodium acetate/citric acid buffer (pH 6.0)	50ml
Hydrogen peroxide 30% w/v	7.5μl

OR

(Sigma® Method)
Dissolve one TMB free base tablet (T5525) in 1ml DMSO.
Add 1ml of TMB-DMSO solution to 9ml phosphate citrate buffer (buffer made by dissolving content of one capsule (P9305) in 100ml of deionized water).

APPENDIX THREE

MANUFACTURERS' AND SUPPLIERS' NAMES AND ADDRESSES

A. Tuck and Sons
Beeches Road
Battle Bridge
ESSEX

BDH Ltd
Broom Road
Poole
DORSET
BH12 4NN

Camlab Ltd
Nuffield Road
CAMBRIDGE
CB4 1TH

Dynatech Laboratories Ltd
Daux Road
Billingshurst
WEST SUSSEX
RH14 9SJ

Flow Laboratories Ltd
Woodcock Hill
Harefield Road
Rickmansworth
HERTS
WD3 1PQ

Gibco Ltd
Unit 4
Cowley Mill Trading Estate
Longbridge Way
UXBRIDGE
UB8 2YG

Glaxovet Ltd
Greenford
MIDDLESEX

Hawksely (Gelman Hawksley & Sons)
10 Harrowden Road
Breckmills
NORTHAMPTON

Heraeus Equipment Ltd
9 Wates Way
Brentwood
ESSEX
CM15 9TB

Leica UK Ltd
48 Park Street
LUTON

May and Baker Ltd
DAGENHAM

Millipore UK Ltd
11-15 Peterborough Road
Harrow
MIDDLESEX
HA1 2IH

MSD-AGVET
Division of Merck Sharp & Dohme Ltd
Hoddesdon
HERTFORDSHIRE
EN1 9BU

MSE Scientific Instruments
Manor Park
Crawley
WEST SUSSEX
RH10 2QQ

Nordic Immunologicals
P.O. Box 544
Maidenhead
BERKS
SC6 2PW

Northumbria Biologicals Ltd
South Nelson Industrial Estate
CRAMLINGTON

Pfizer Ltd
Sandwich
KENT

Scientific Supplies Co.
Scientific House
Vine Hill
LONDON
EC1 5EB

SDS Lavender Mill
Manea
CAMBRIDGE
PE15 0LU

Sigma Chemical Co Ltd
Fancy Road
Poole
DORSET

Sterilin Ltd
Sterilin House
Clockhouse Lane
Fettham
MIDDLESEX

TAAB Laboratories Equipment Ltd
Unit 3 Minerva House
Calleva Industrial Park
Aldermaston
Reading
BERKS
RG7 4QW

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P.O. Box 6
Twyfords
Reading
BERKS
RG10 9NL

APPENDIX FOUR

PUBLICATIONS

Some aspects of this work were presented and published as follows:

Fakae, B.B., Harrison, L.J.S. and Sewell, M.M.H. (1993). Effect of conjoint infection with *Trypanosoma congolense* on protection against challenge infection with *Heligmosomoides polygyrus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**:120-121

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Effect of conjoint infection with *Trypanosoma congolense* on protection against challenge infection with *Heligmosomoides polygyrus*

B. B. Fakae, L. J. S. Harrison and M. M. H. Sewell *Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian, EH25 9RG, UK*

Conjoint nematode and trypanosome infections are common in sub-Saharan Africa. As a preliminary step to studying aspects of such conjoint infections in the ruminants, a mouse model was developed in which it was possible to investigate the effect of conjoint *Trypanosoma congolense* and *Heligmosomoides polygyrus* infection on the ability of the mice to resist a secondary nematode challenge.

Female out-bred TO mice were immunized by methods which gave either 50% or 100% protection and later challenged either singly with 500 infective larvae of *H. polygyrus* or were conjointly infected with *H. polygyrus* and 10^4 stabilated *T. congolense* (TREU 1881). Weekly differential counts of leucocytes were performed on Giemsa-stained blood films from each animal. The mortality of the mice was charted and the worms in the survivors were counted at post-mortem examination, 30 d after the challenge infection.

The conjointly infected mice were severely compromised, 50–100% dying. This suggests a synergistic interaction between these parasites. However, this synergy was reduced in mice resistant to *H. polygyrus*. The differential leucocyte counts showed that the conjoint infection with the trypanosome suppressed the eosinophilia characteristically associated with immunity against the nematode infection.